

What efficacy and safety profiles can we expect

*Mathematical and Computational Biology in Drug Discovery
(MCBDD) Module IV*

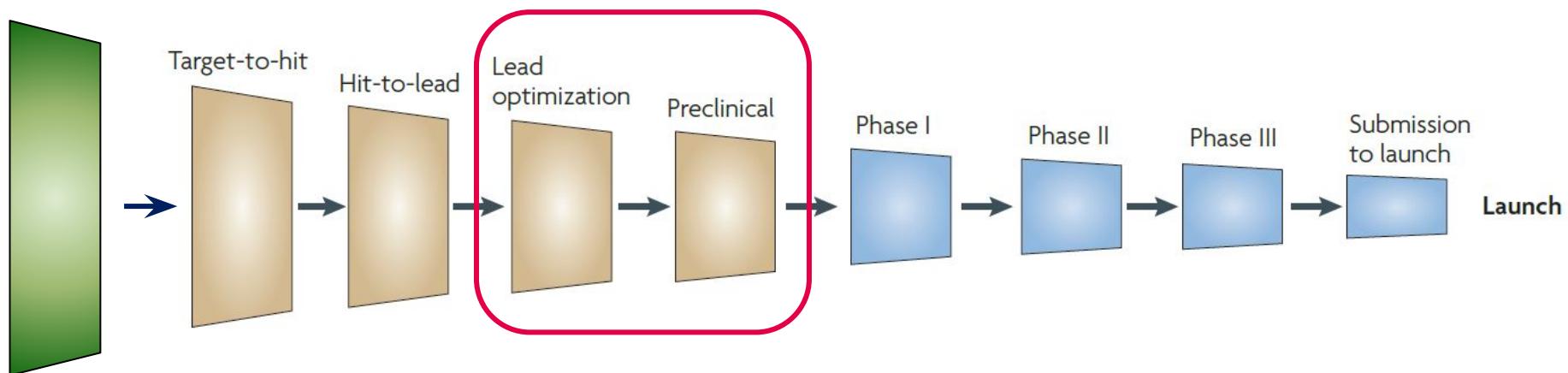
*Dr. Jitao David Zhang
April-May 2023*

Outline of Lecture 9

- Understanding pharmacology and toxicology with *in vitro*, *in vivo*, and *in silico* models
- Cell-type specific response to drugs
- Single-cell RNA sequencing for disease understanding and drug discovery

Where are we now

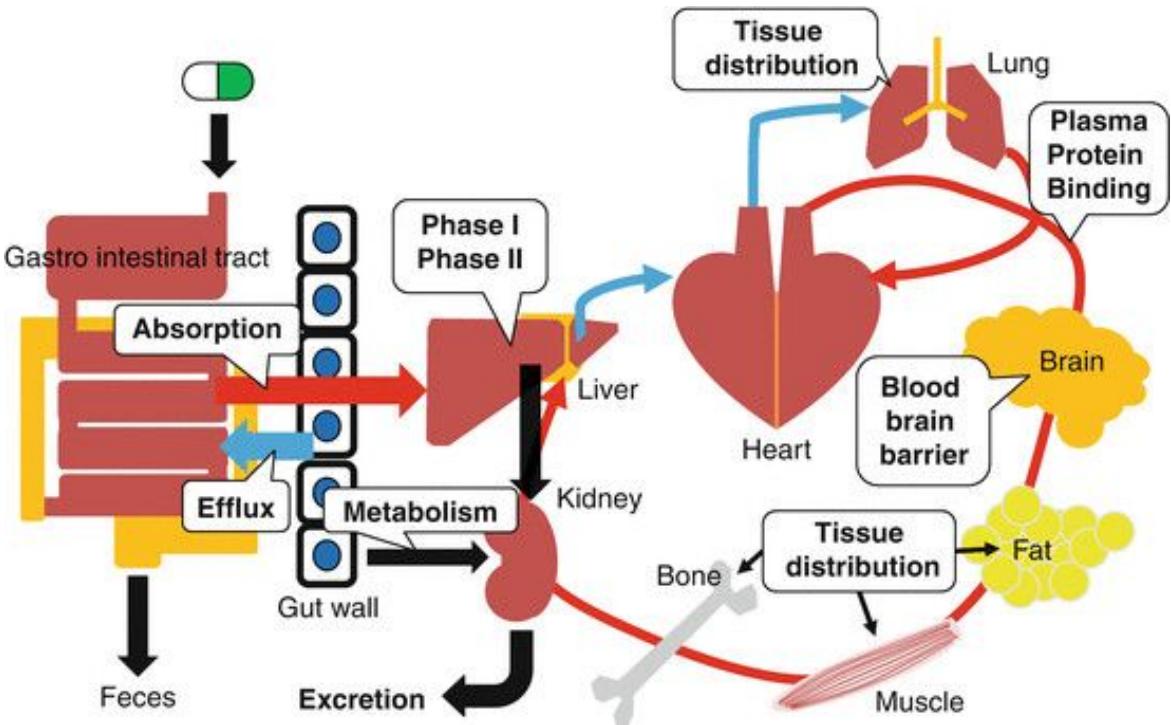
Target identification & assessment



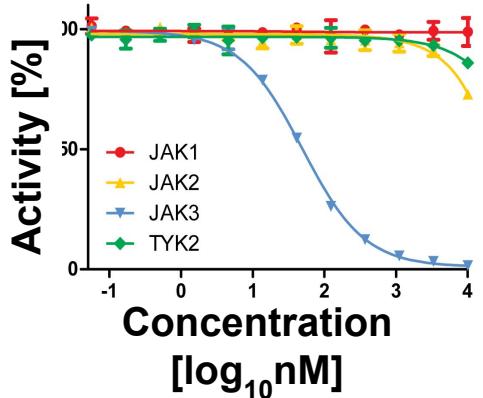
Goal: we want to select **one compound** from a few (~ 10^2 - 10^0) for entry in human.

Factors that affect efficacy and safety profiles

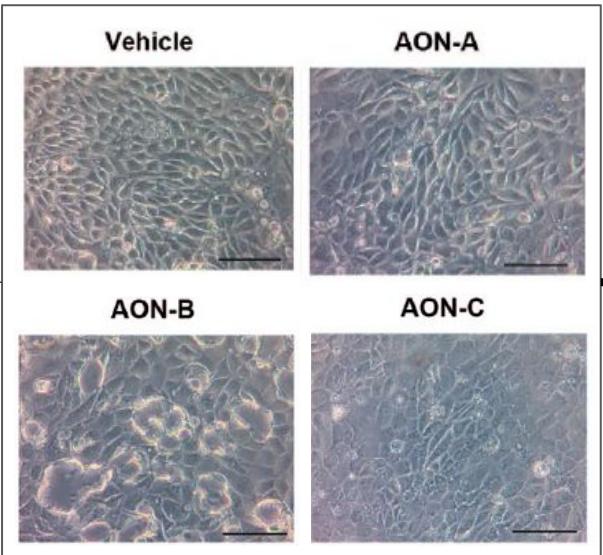
- Absorption
- Distribution
- **Pharmacology**
- **Toxicology**
- Metabolism
- Excretion



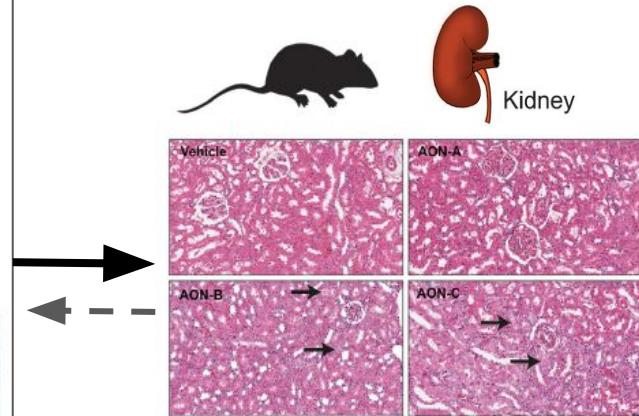
Classical workflow of efficacy and toxicity assessment



Biochemical &
biophysical assays



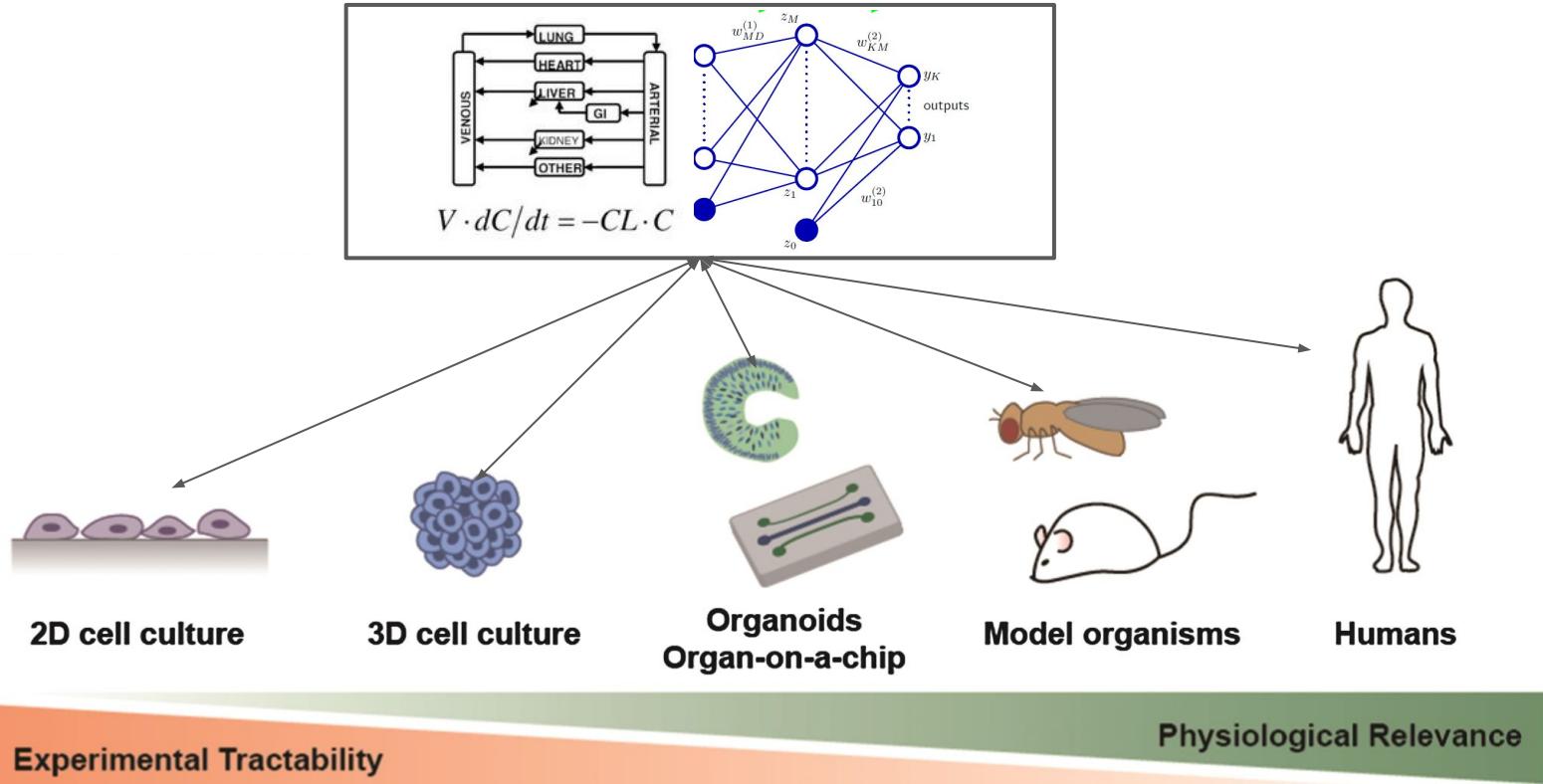
Cellular assays
(*in vitro*)



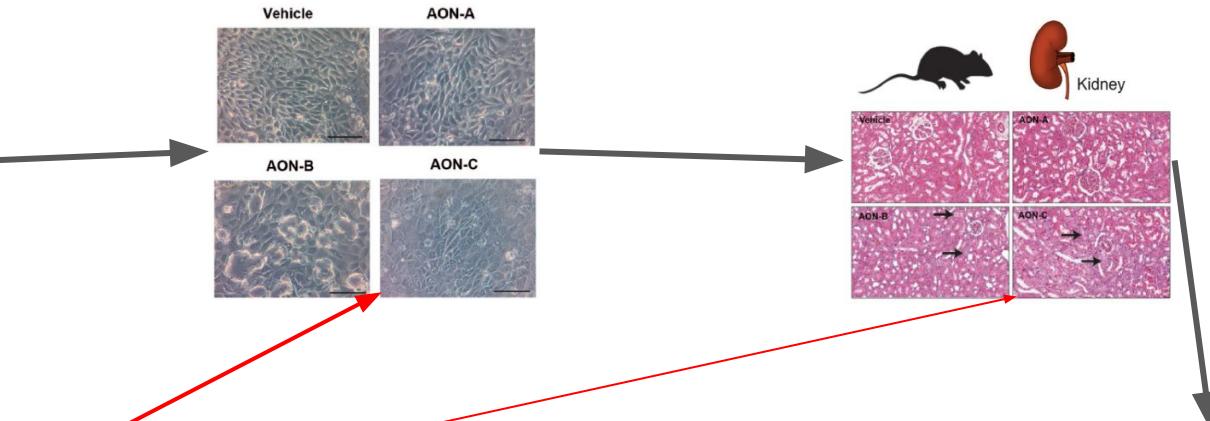
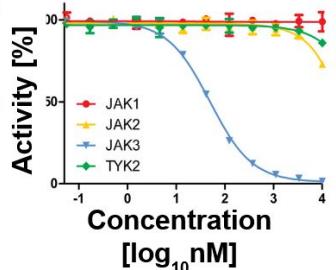
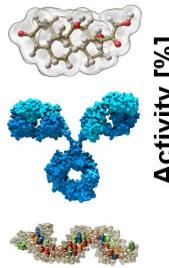
Animal
experiments
(*in vivo*)

→ Usual workflow
 ← - - Assay development

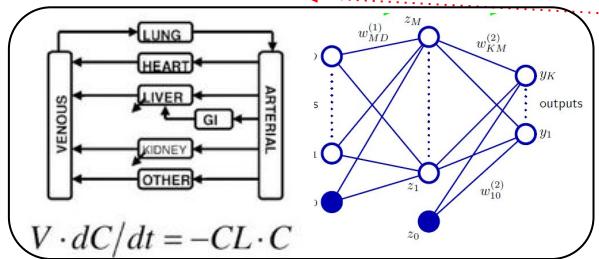
Biological and computational models of human diseases



Computational methods empower efficacy and toxicity assessment



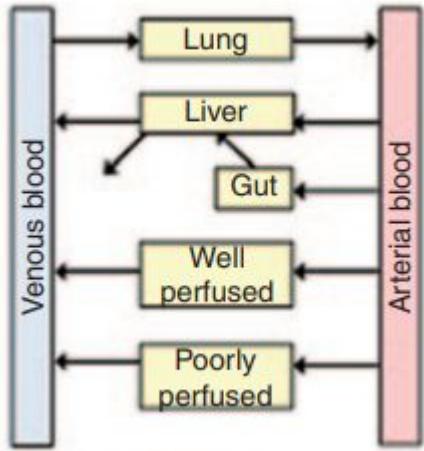
High-throughput
technologies (omics,
microscopy, etc.)



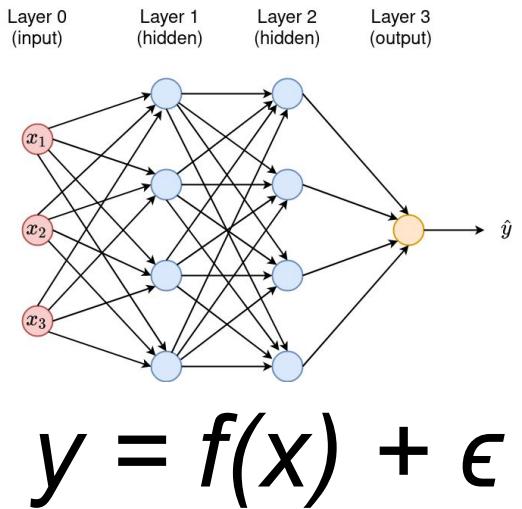
Mechanistic, causal,
and statistical models



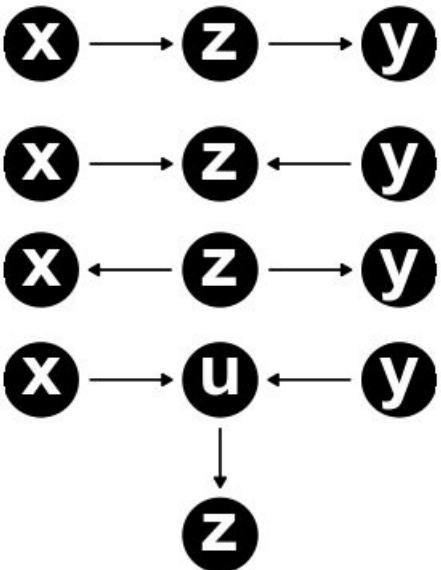
Three types of computational models



Mechanistic models

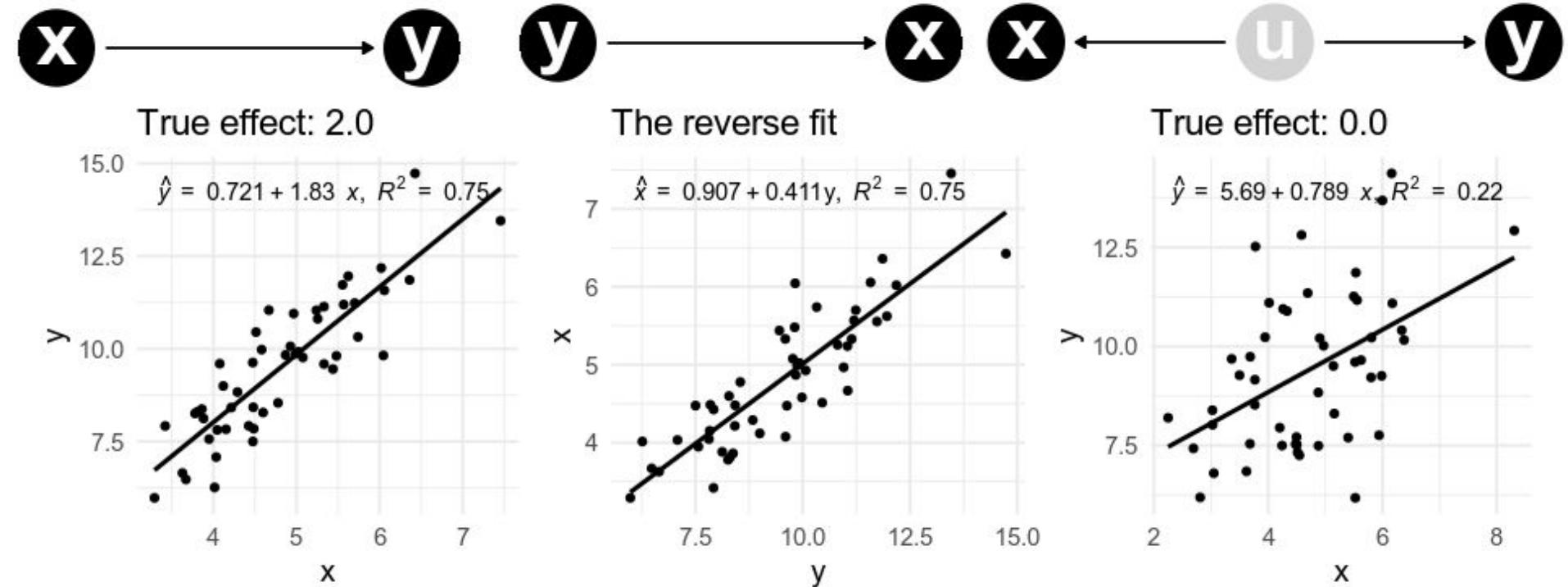


Statistical and
machine-learning models



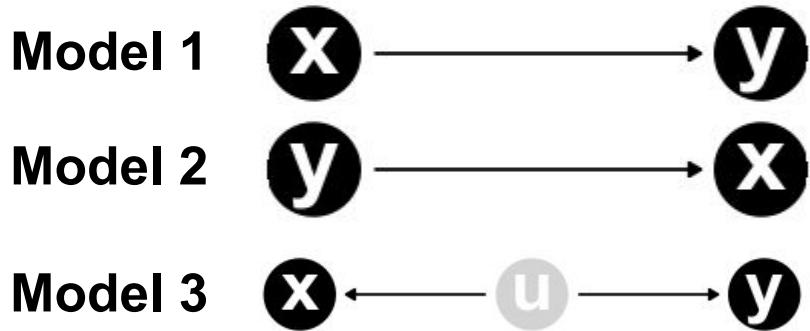
Causal models

Correlation is caused by causation, confounding, coincidence, or conspiracy



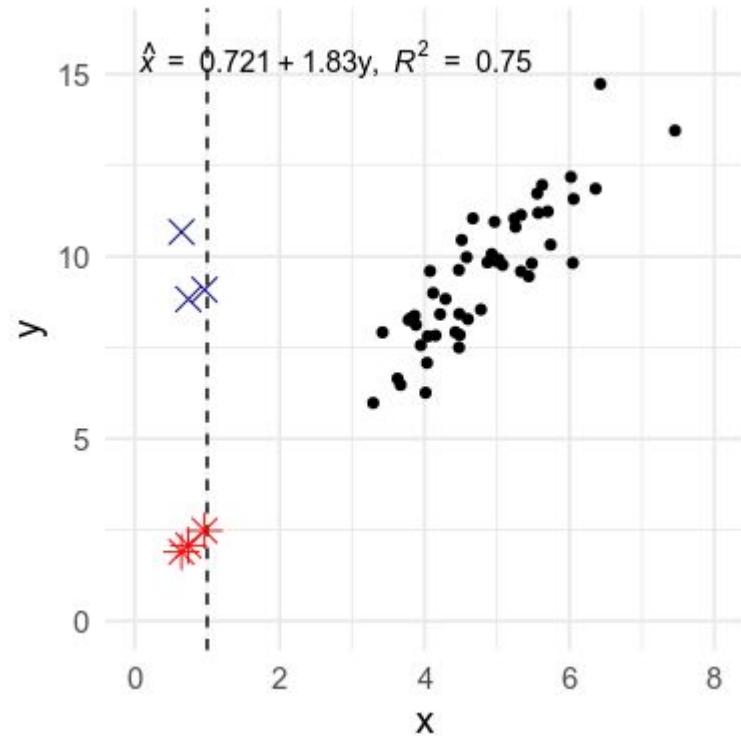
Statistical models alone cannot derive causality from correlation

We learn causality by (1) listing models explicitly and (2) manipulating a variable and observe the outcomes

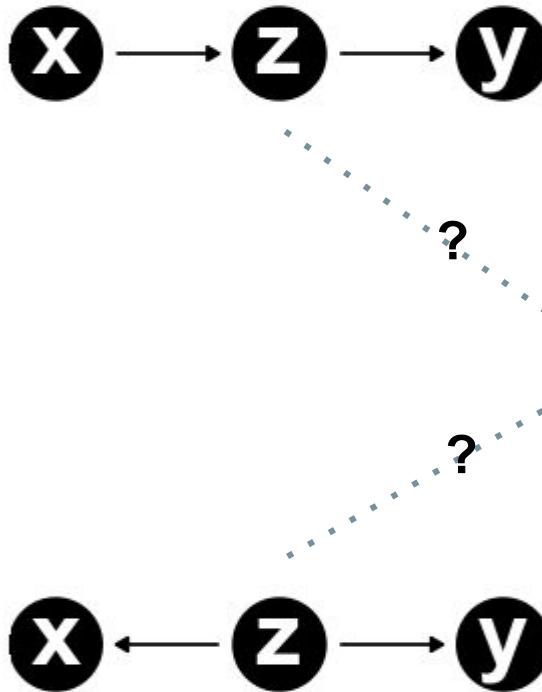


Assume that the data is generated by either Model 1, or Model 2, or Model 3. And assume that we can manipulate the value of X by setting it to 1.0 (the dash line).

Question: which outcomes (red stars or blue crosses) would support which models? Why?

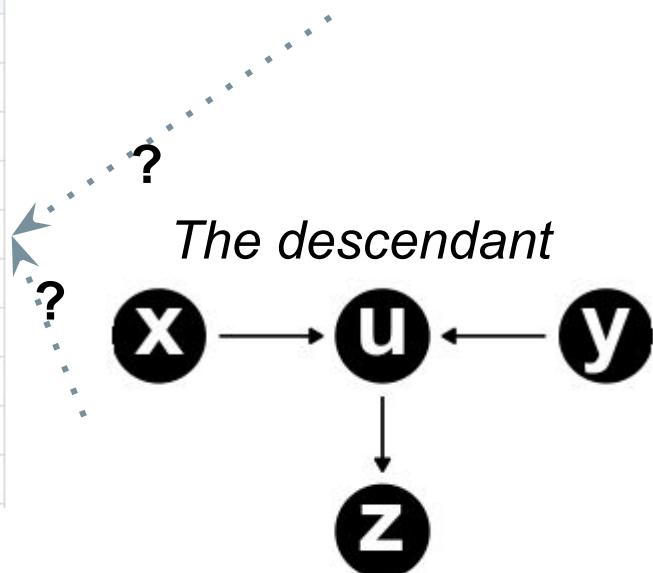


Causality is crucial for drug discovery



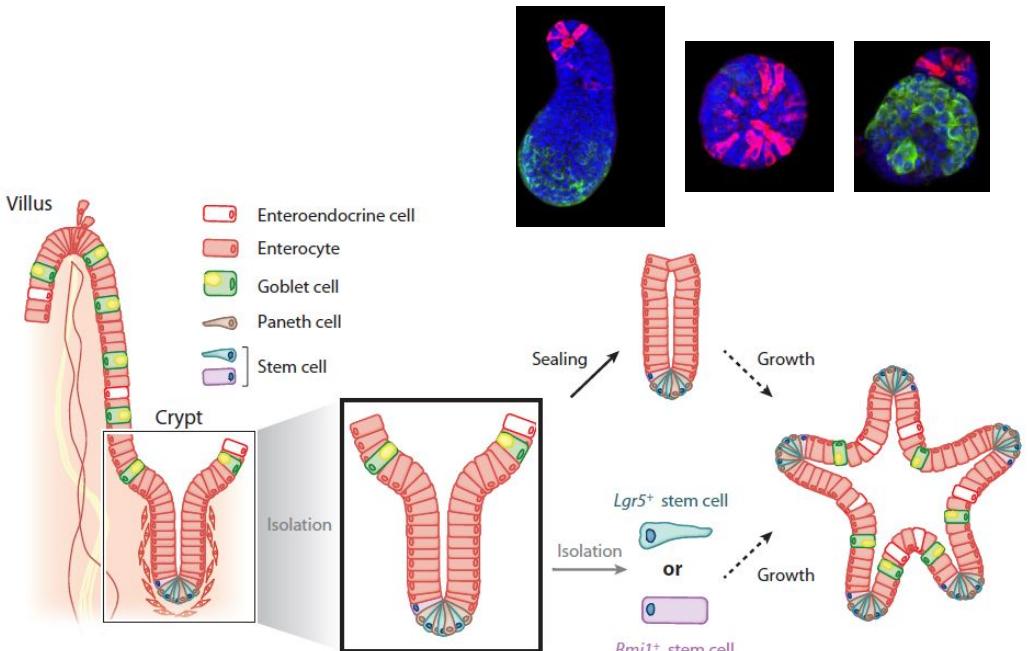
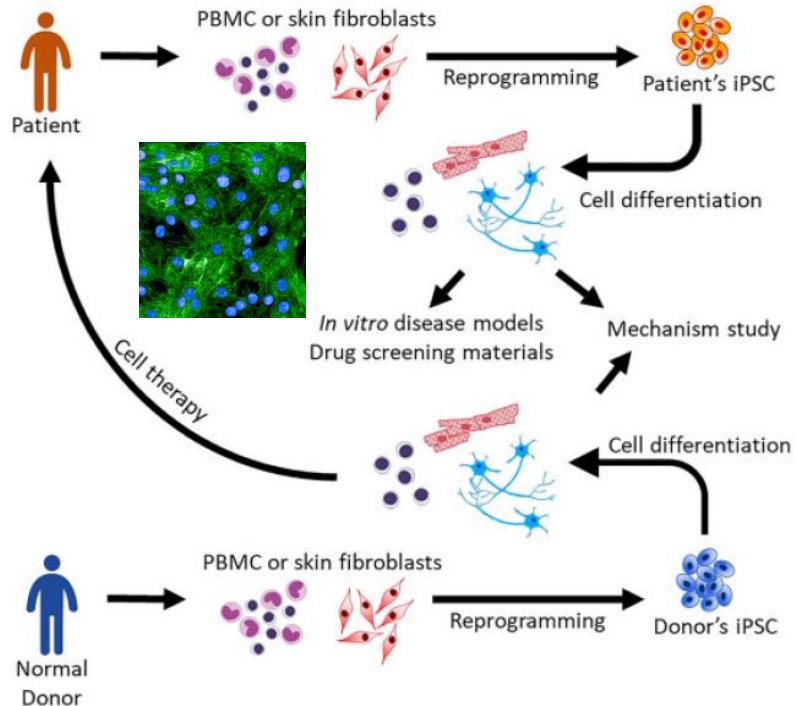
Biomarker, tox study, pathology,
omics data, real-world data, ...

	x	z	y
1	0.835386320	1	-0.73897252
2	-0.005354014	-1	-0.82972315
3	0.058788286	1	0.76213369
4	-1.015602246	-1	-0.05951719
5	-0.339569780	-1	-0.11745910
6	-0.041077979	-1	-1.28243716
7	0.363740407	1	-0.30570762
8	0.119496314	-1	-1.19932461
9	0.257108454	-1	-1.06044066
10	0.304537158	-1	-0.43396492



We need both models (knowledge + assumptions) and data to infer causality.

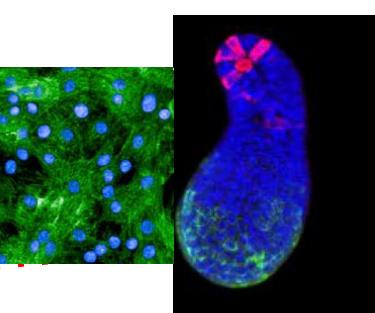
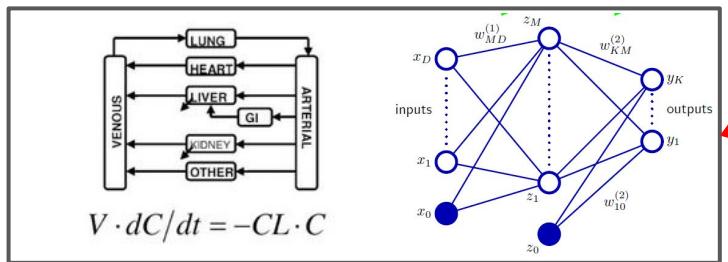
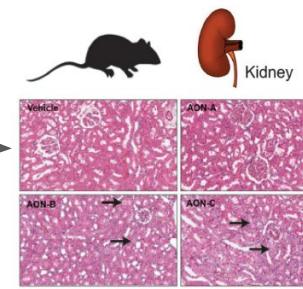
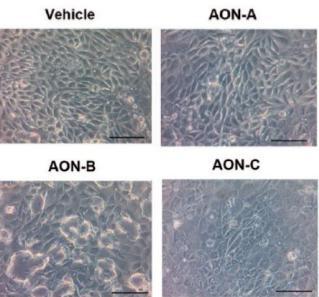
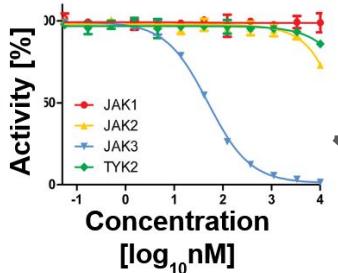
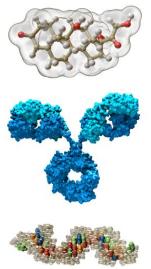
Stem cells and organoids empower efficacy and toxicity assessment



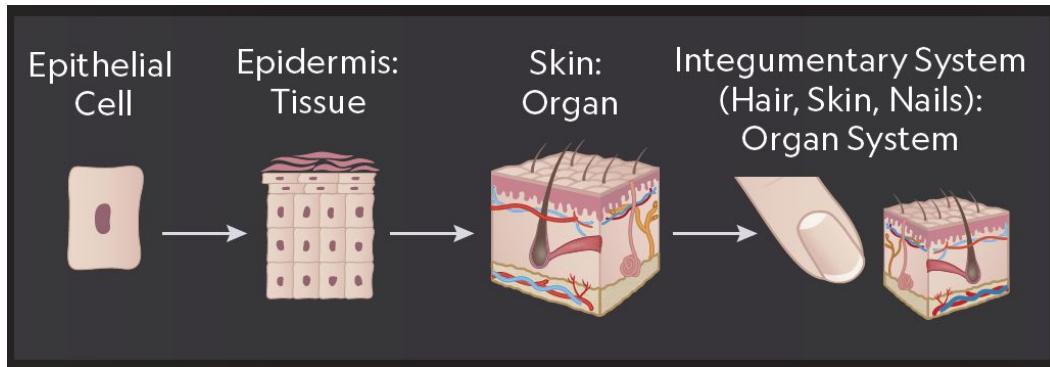
Small-intestinal organoids

Induced pluripotent stem-cells

Computational methods and novel biological models empower efficacy and toxicity assessment



Complexity Increases Through a System

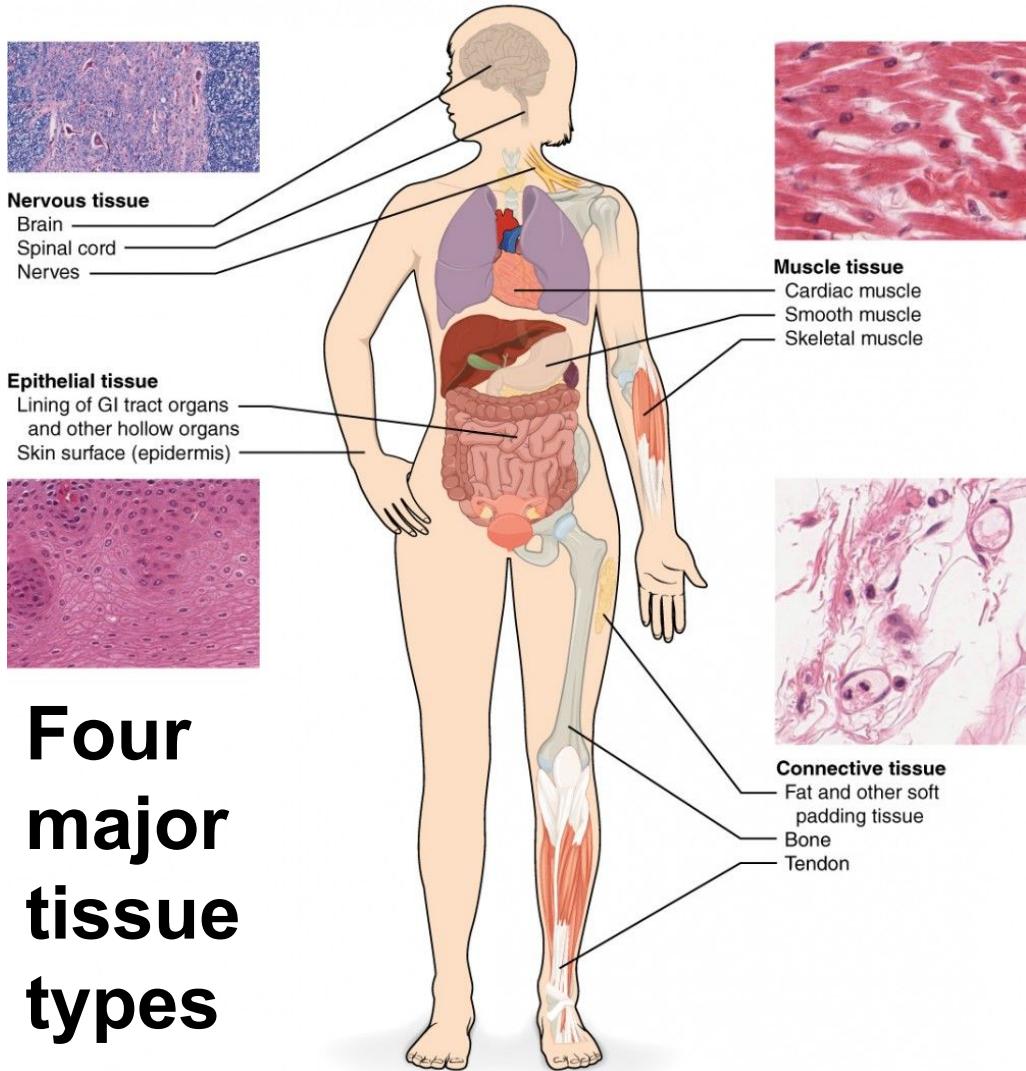
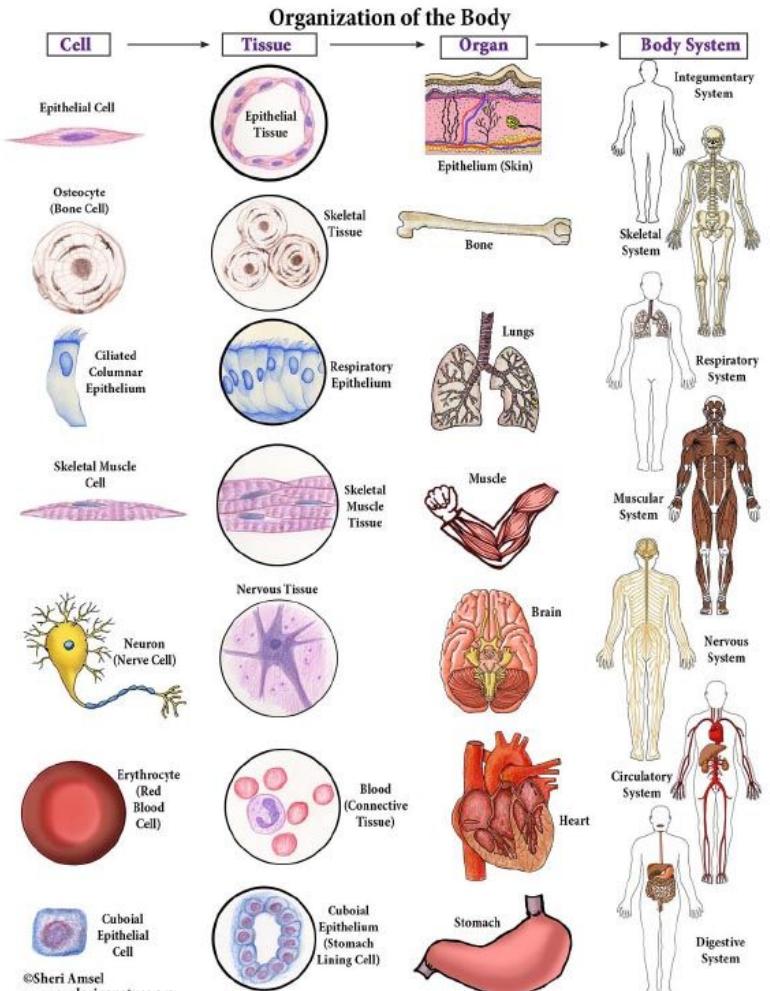


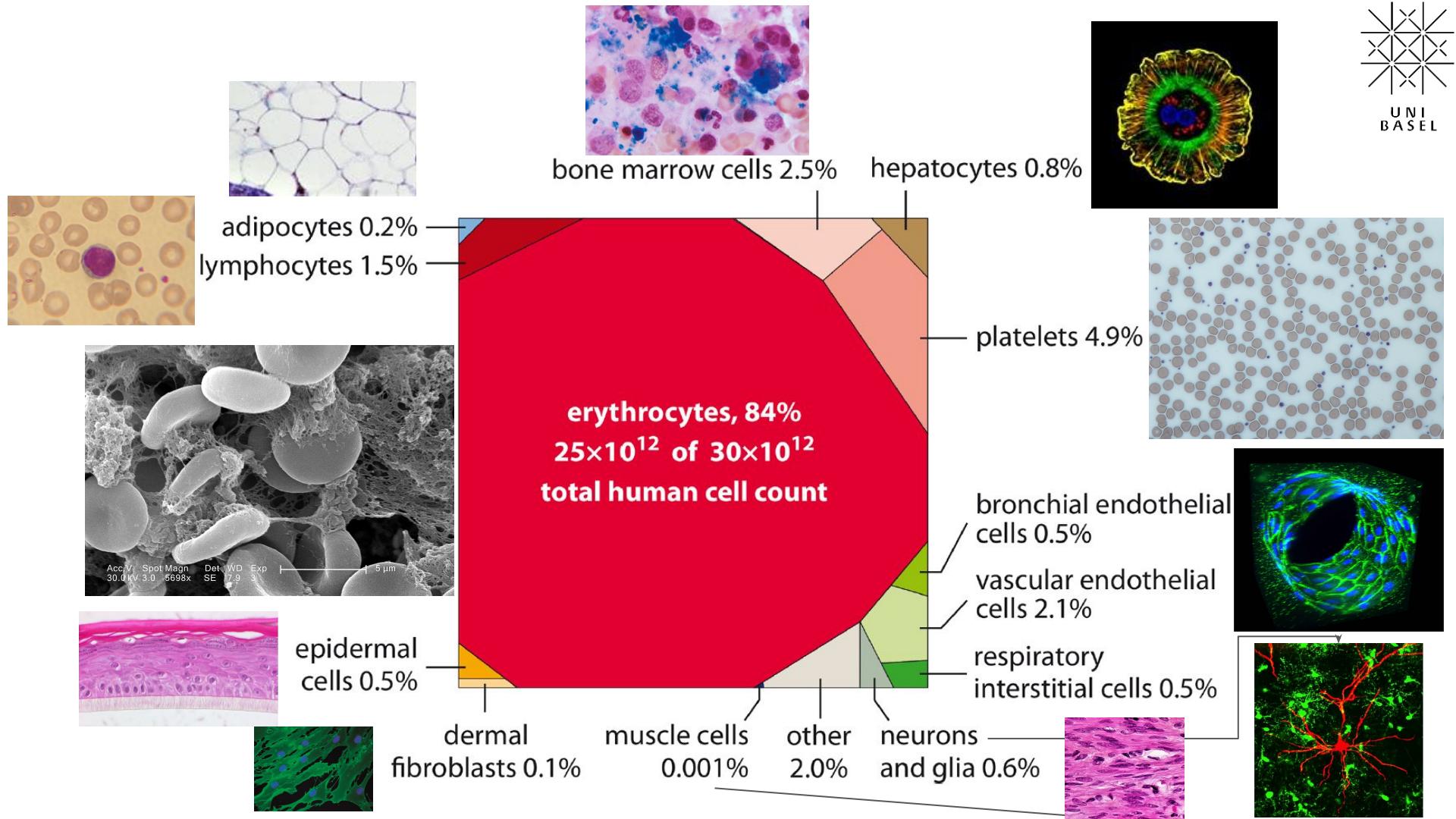
Cells: basic building blocks, variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues





What's in a drop of blood? Ask a doctor or a biologist!

Plasma:

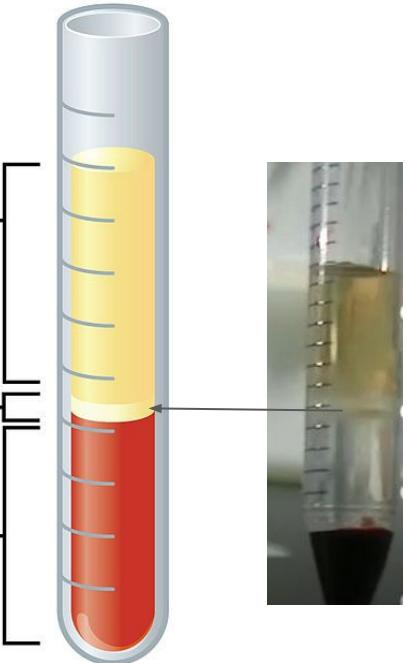
- Water, proteins, nutrients, hormones, etc.
- ~55%

Buffy coat:

- White blood cells, platelets
- <1%

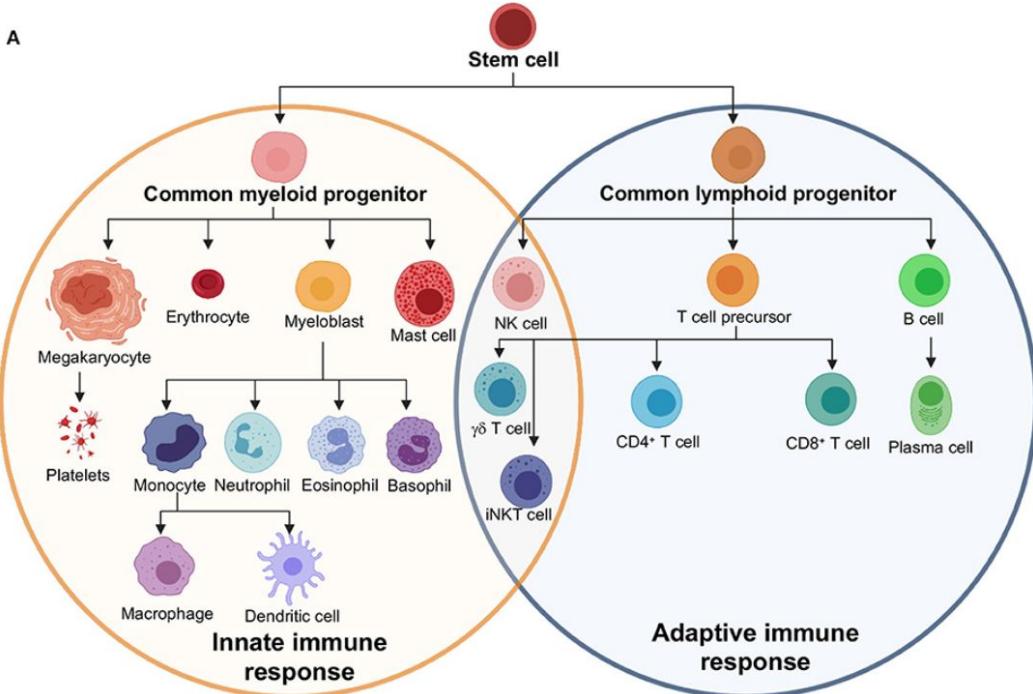
Hematocrit:

- Red blood cells



Normal Blood:

♀ 37%–47% hematocrit
 ♂ 42%–52% hematocrit

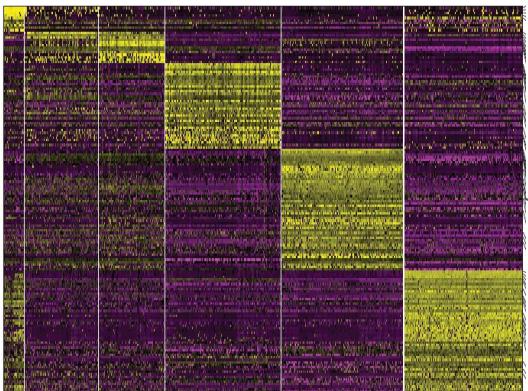


What's in a drop of blood? Count the genes!



Sequencing

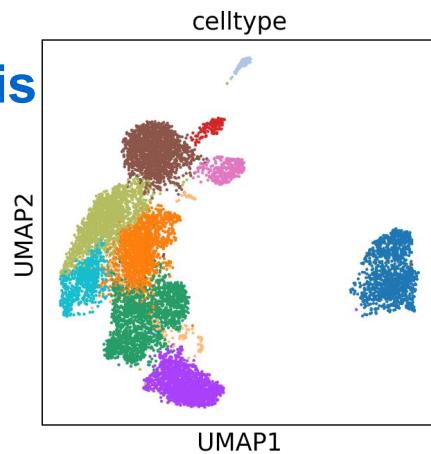
Genes



Cells

Low Expression  High Expression

Data analysis

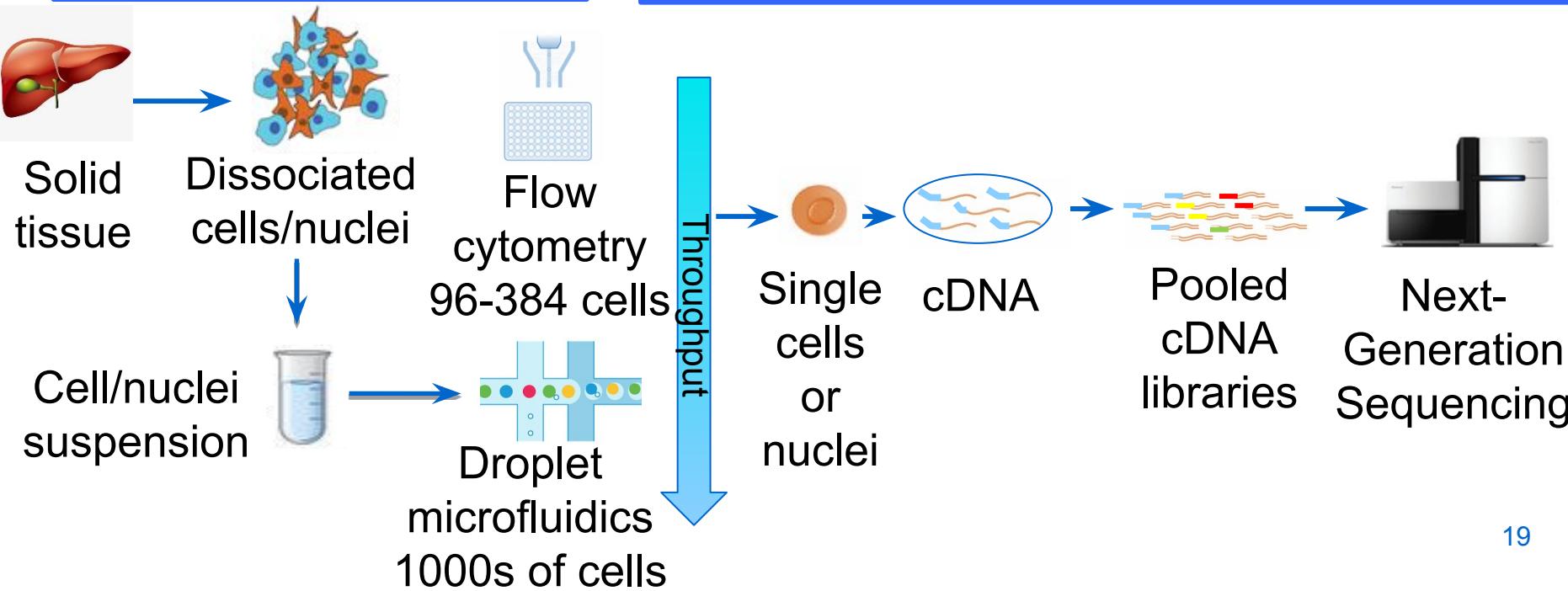


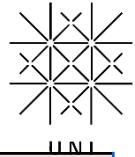
- B-cell
- CD4 T-cell
- CD8 T-cell
- DC
- NK cell
- monocyte CD14+
- monocyte CD16+
- naive CD4 T-cell
- naive CD8 T-cell
- pDC
- unknown

Single-cell sequencing (scSeq) workflow

Tissue dissociation

Single cell capture and transcriptome sequencing





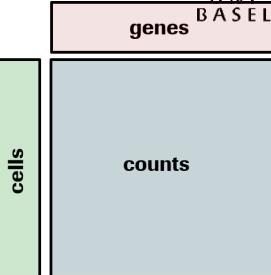
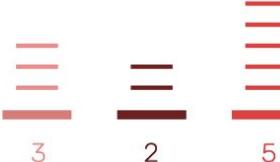
A linearized workflow of scSeq data analysis

From short reads to gene-cell matrix

Alignment

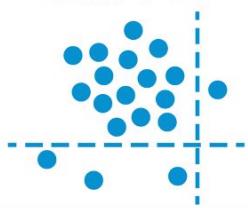


Quantification

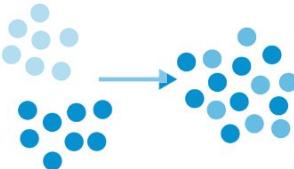


QC, filtering & normalization,
dimensionality reduction, and
clustering

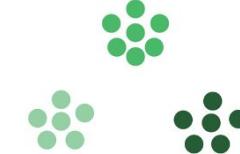
Quality control



Normalisation



Clustering



Downstream analysis

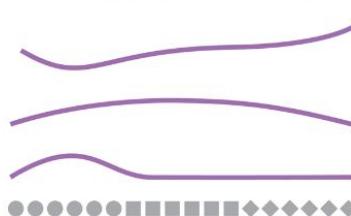
Differential expression



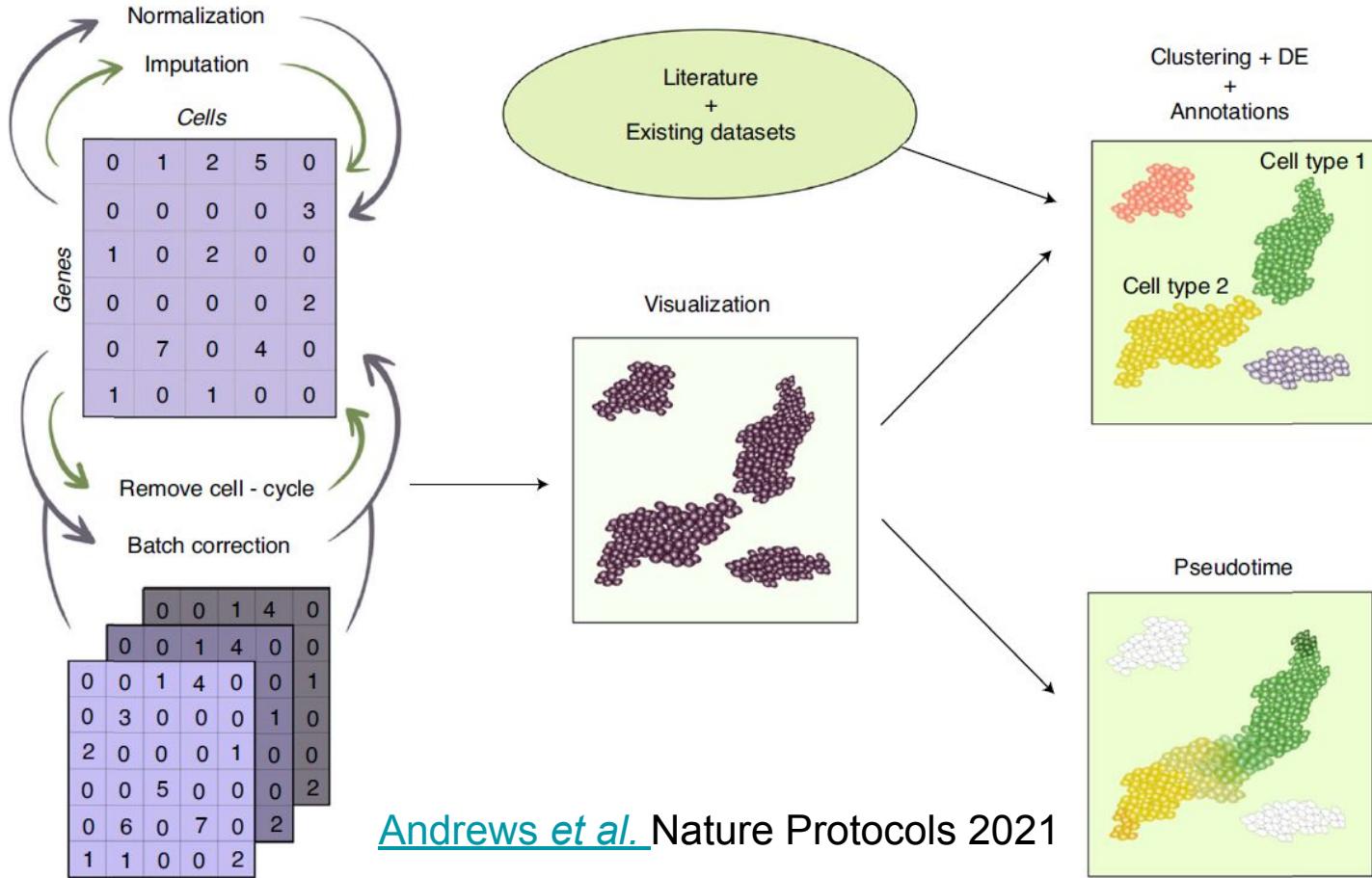
Marker genes



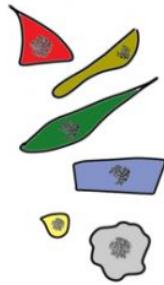
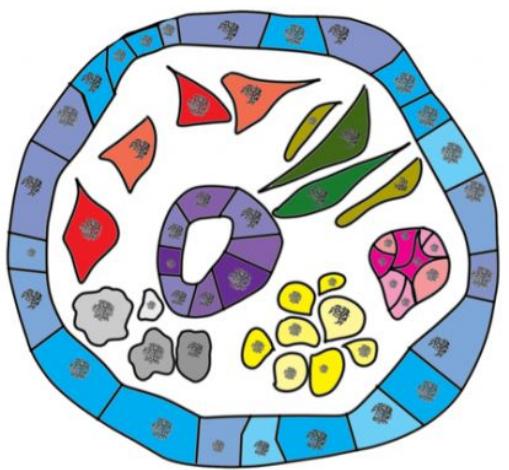
Expression patterns



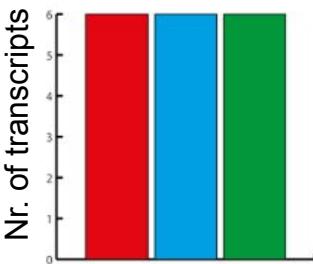
Overview of the computational workflow



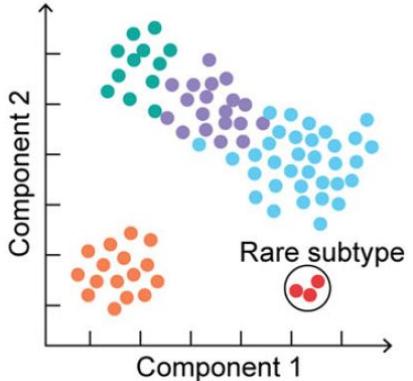
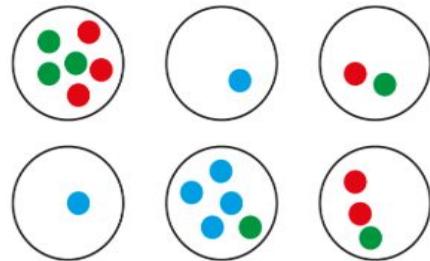
Single-cell biology benefits both disease understanding and drug discovery



Bulk analysis

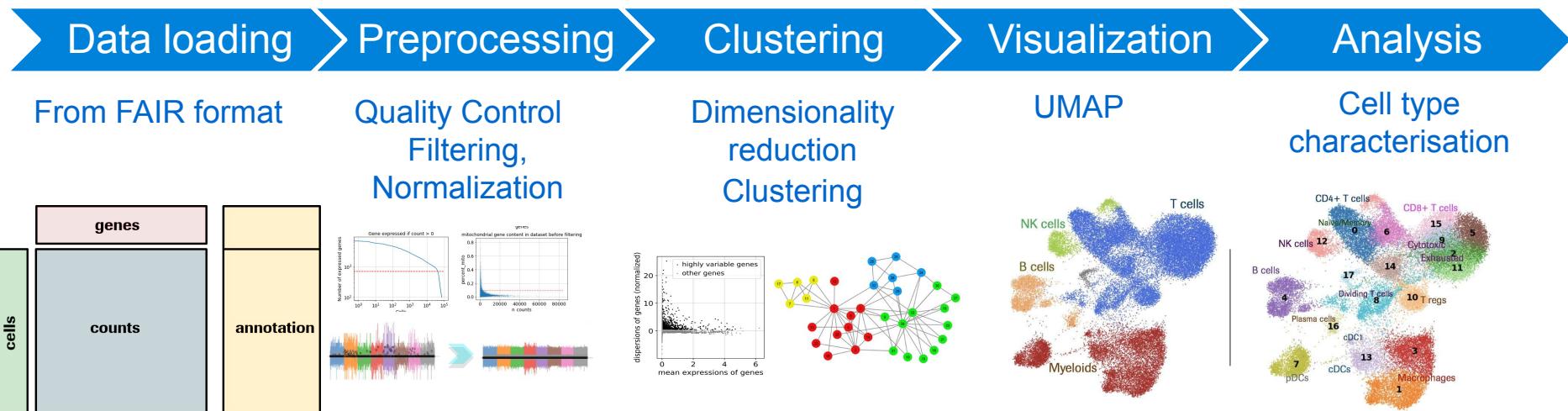


Single cell transcriptome analysis



BESCA: An open-source Python package for single-cell gene expression analysis

An automated standard workflow

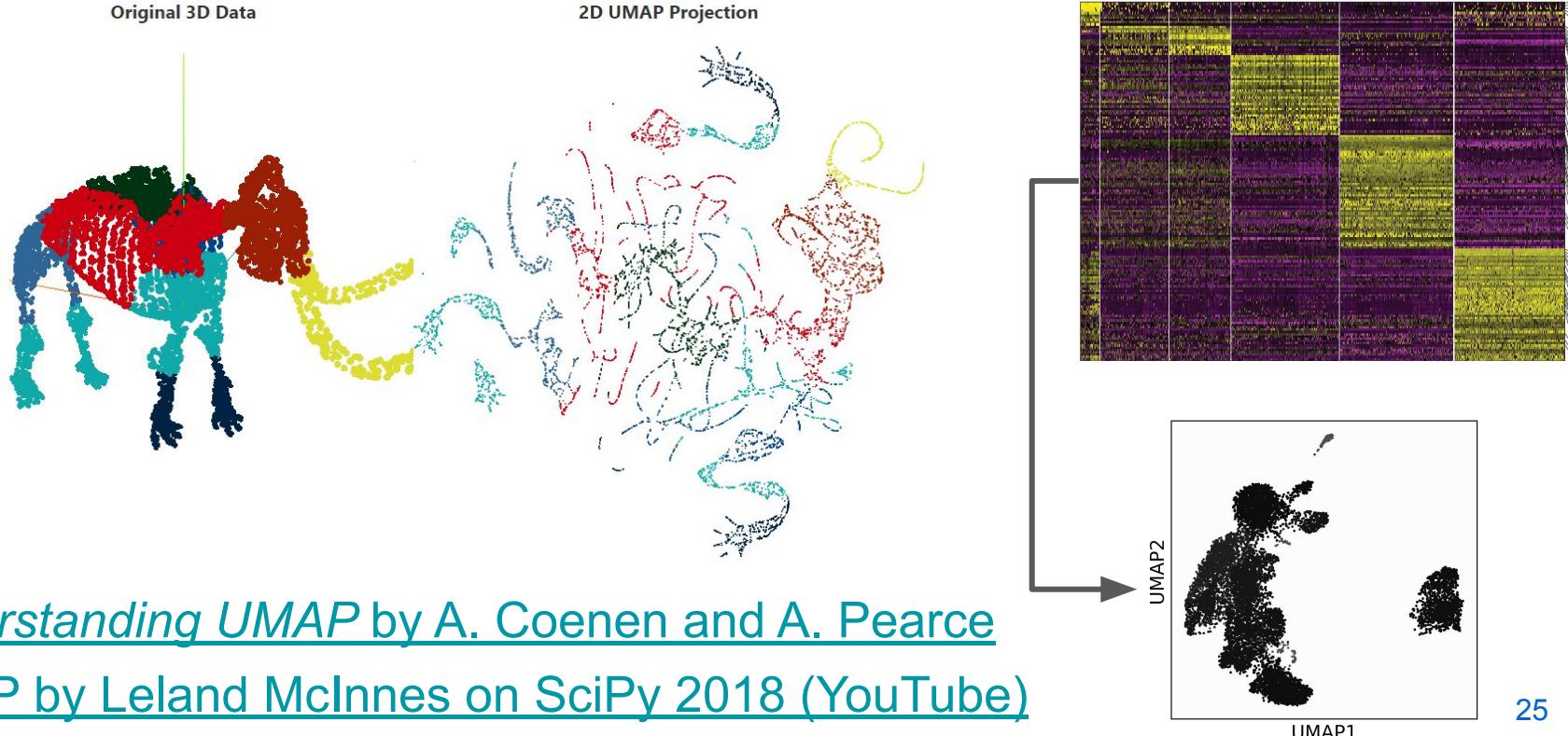


How to represent voxels with pixels?



The elephant bull *Tusker* at Zolli Basel plays with a tree trunk on a post (2022)

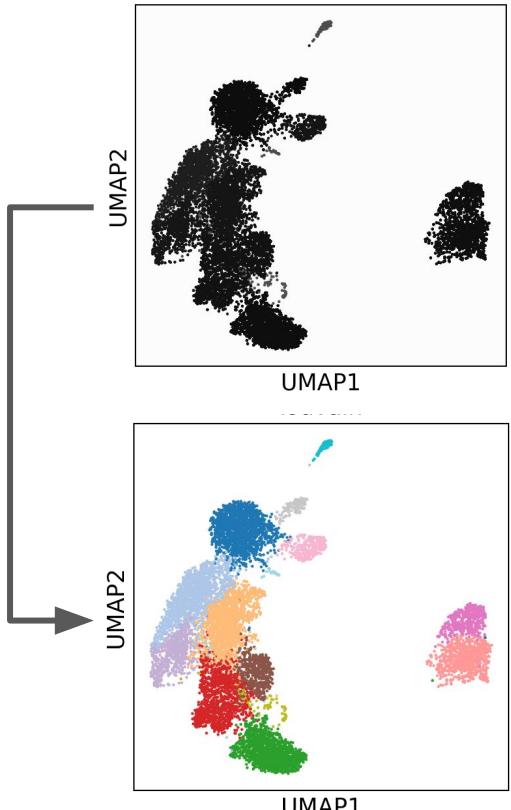
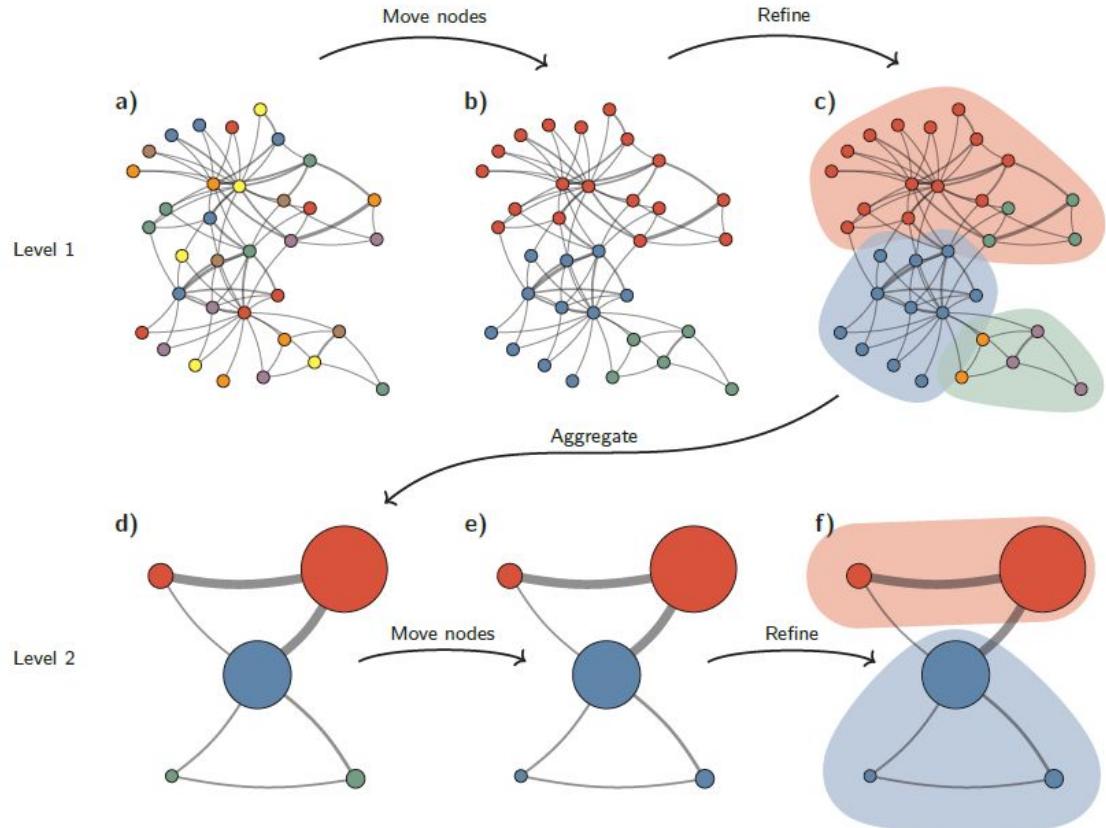
Uniform Manifold Approximation and Projection (UMAP) for dimension reduction



[Understanding UMAP](#) by A. Coenen and A. Pearce

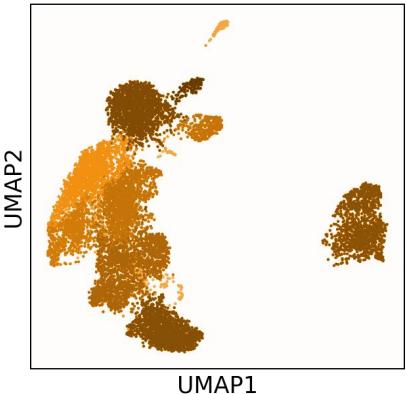
[UMAP](#) by Leland McInnes on SciPy 2018 (YouTube)

The Leiden Algorithm for Community Detection

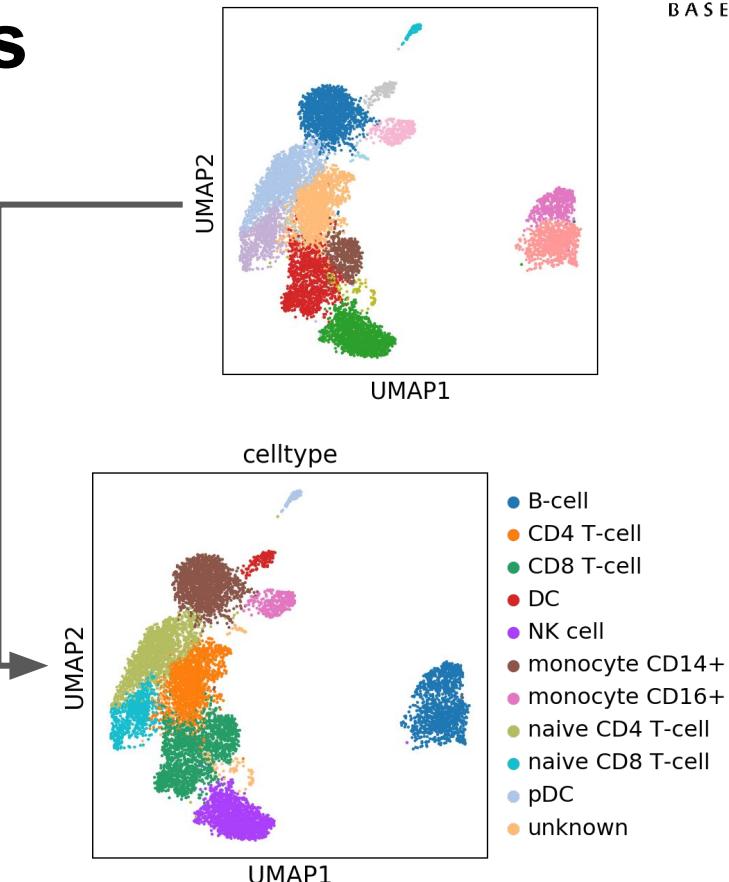


Biological knowledge and visual inspection is used to annotate cell types

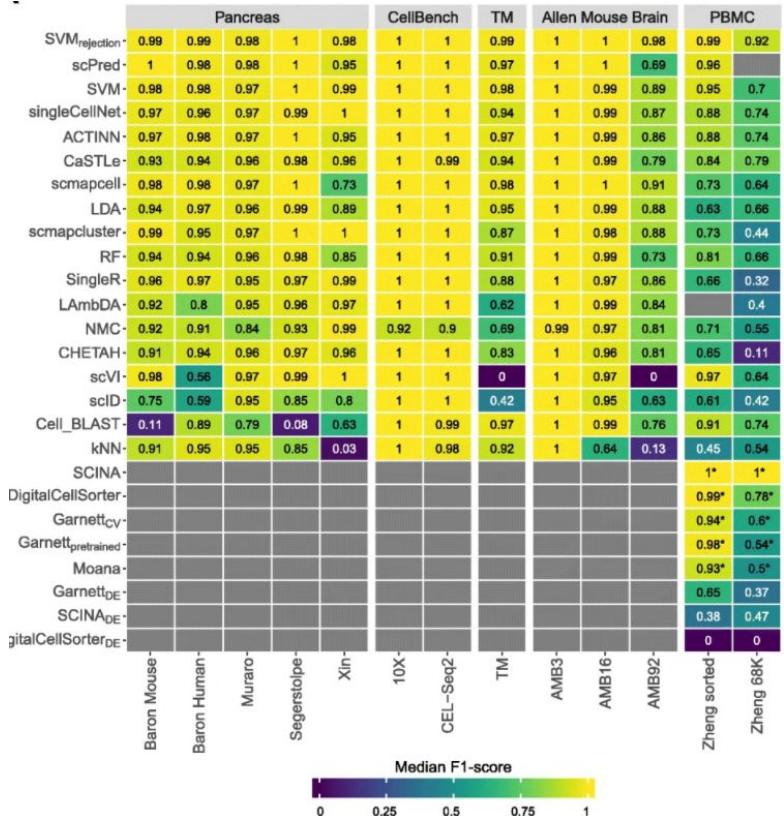
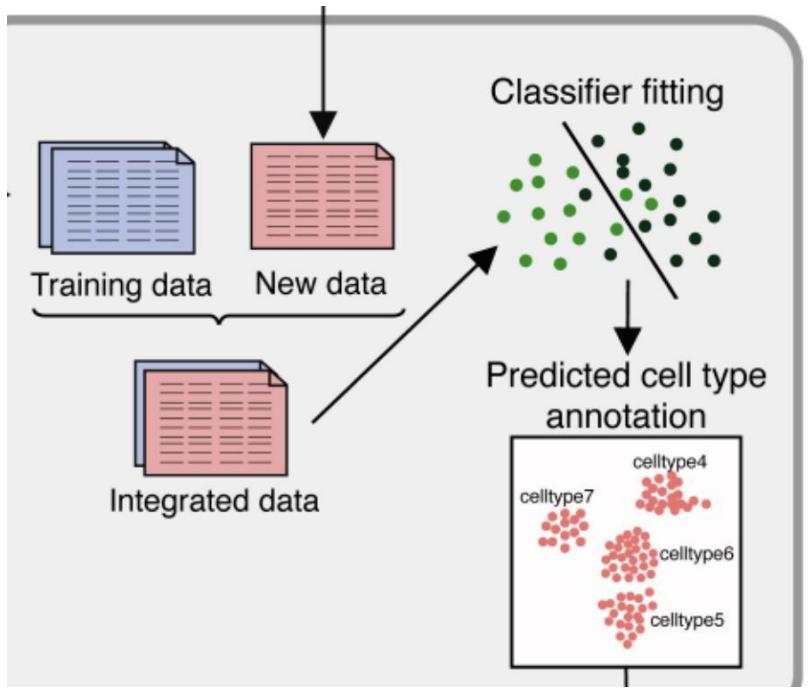
Heatmap
of gene X



lymphocyte	PTPRC							
myeloid	S100A8	S100A9	CST3					
Bcell	CD19	CD79A	MS4A1					
Tcells	CD3E	CD3G	CD3D					
CD4	CD4							
CD8	CD8A	CD8B						
NKcell	NKG7	GNLY	NCAM1					
monocyte	CST3	CSF1R	ITGAM	CD14	FCGR3A	FCGR3B		
macrophage	CD14	IL1B	LYZ	CD163	ITGAX	CD68	CSF1R	FCGR3A

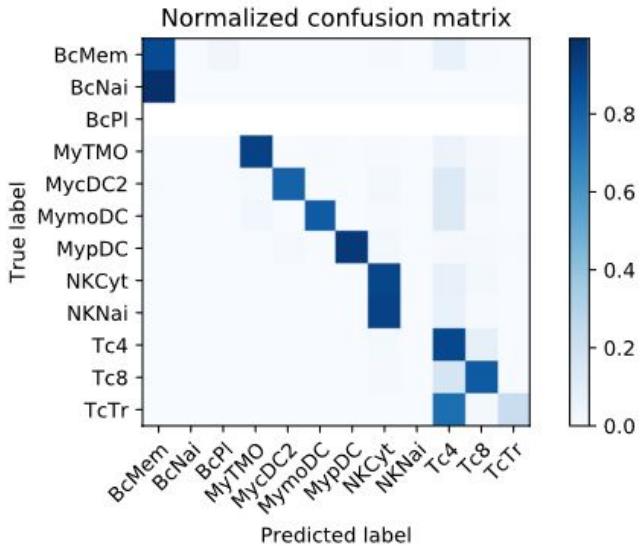
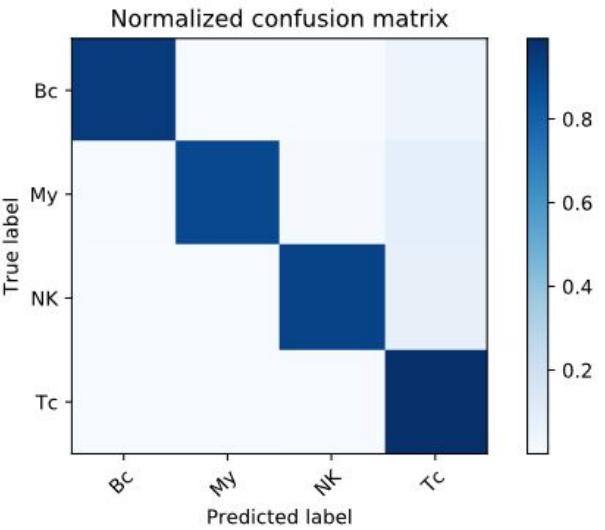
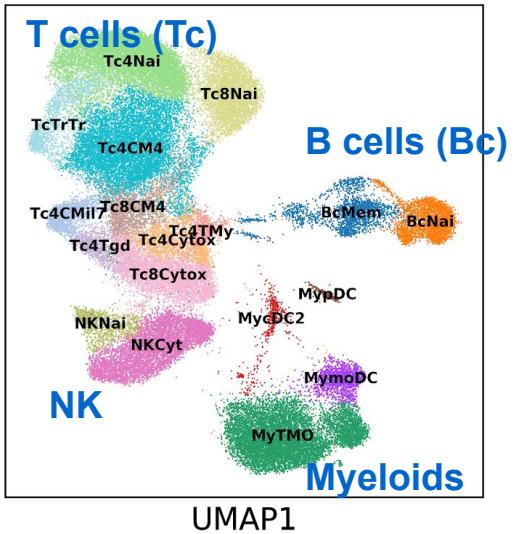


Cell type annotation with machine learning



A PBMC example of cell type annotation

UMAP2



- Broad level cell types, including B cells (Bc), Myeloid (My), NK cells (NK) and T cells (Tc), are successfully predicted.
- Missing and highly similar cell types cause challenges with increased granularity. Essential: reference data quality and knowledge of cell types. ²⁹

Single-cell biology is important in drug discovery

Disease understanding:

disease-specific cell types
and states



Target identification:

expression pattern in
health and disease across
cell types



Biomarker and patient stratification:

which genes should we measure
in which cell type(s)?



MoA and safety

modelling: perturbation
effect at single-cell level



End of Lecture 8

We are living ecosystems

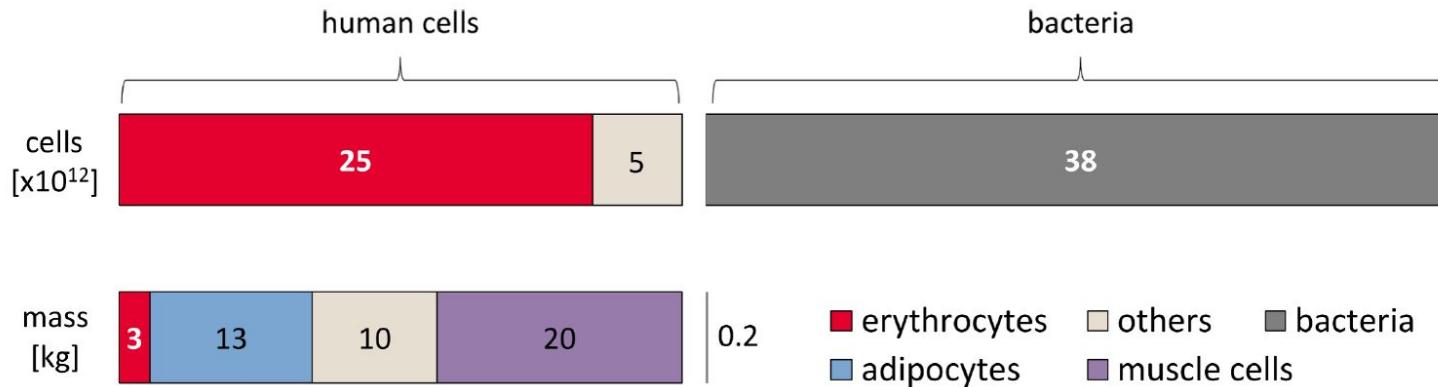
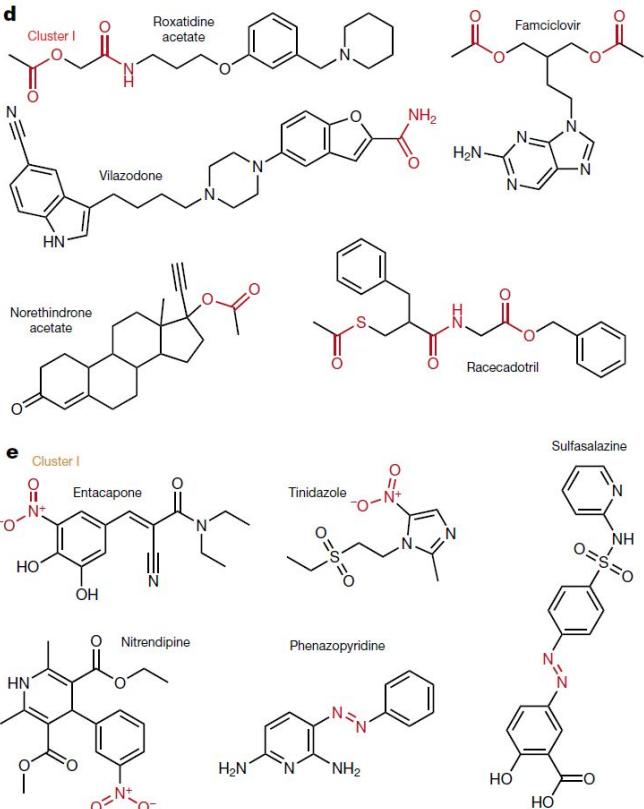
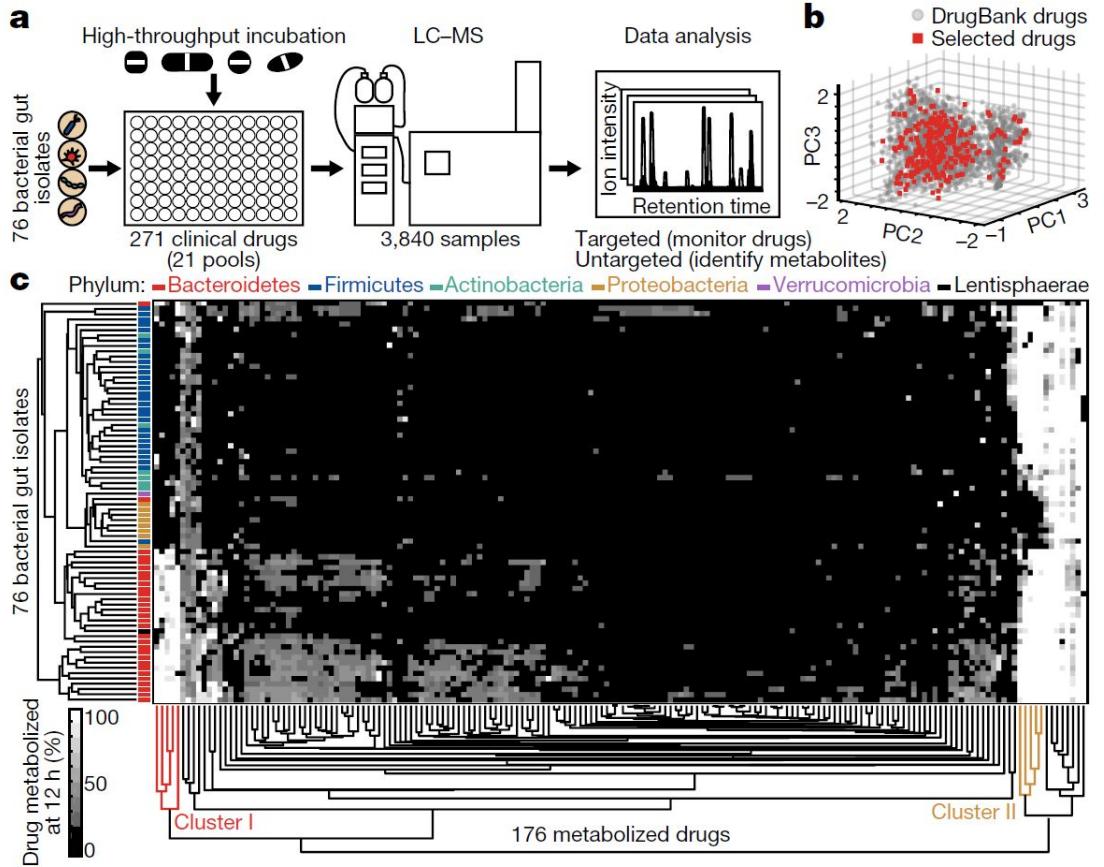


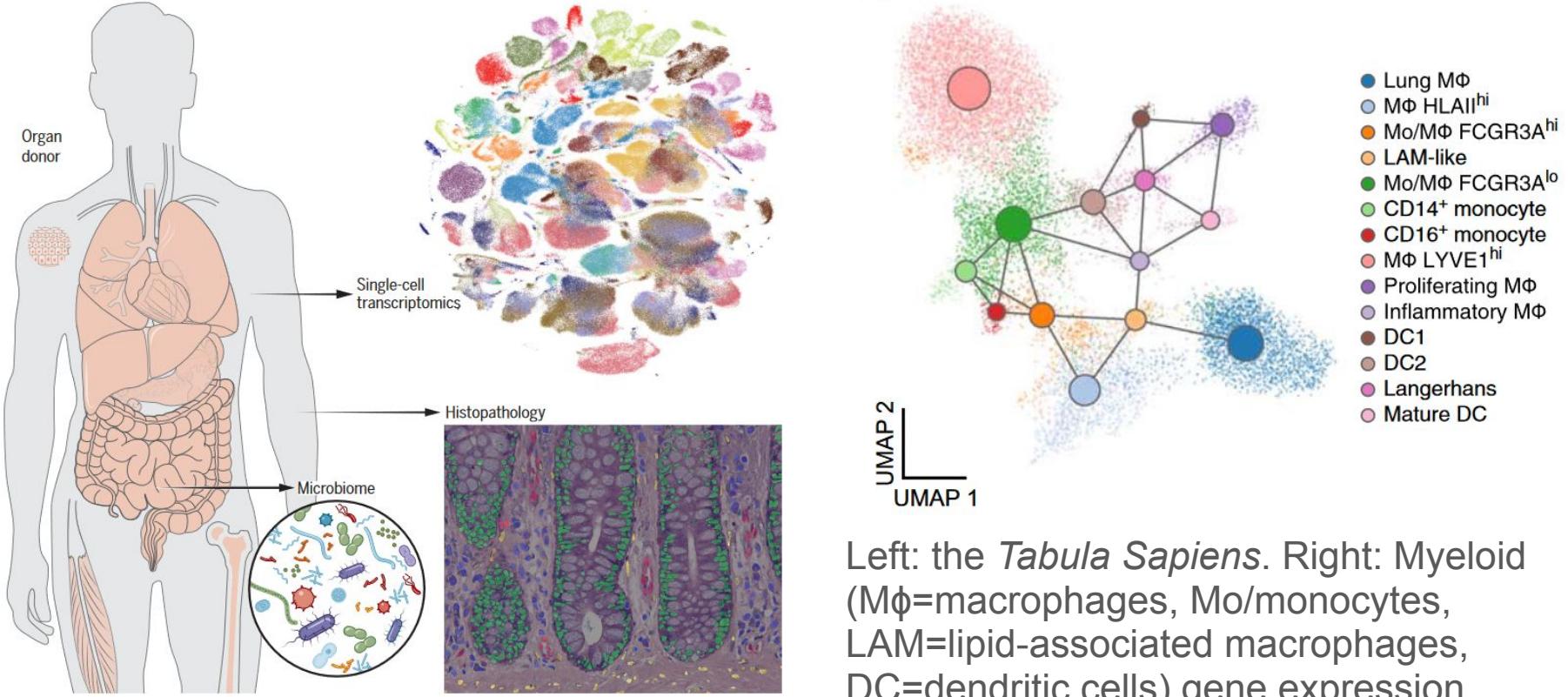
Table 3. B/H ratio for different population. See Table B in [S1 Appendix](#) for full references.

population segment	body weight [kg]	age [y]	blood volume [L]	RBC count $[10^{12}/L]$	colon content [g]	bac. conc. $[10^{11}/g \text{ wet}]^{(1)}$	total human cells $[10^{12}]^{(2)}$	total bacteria $[10^{12}]$	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	1.3
ref. woman	63		3.9	4.5	480	0.92	21	44	2.2
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	2.3
infant	9.6	1	0.8	4.5	80	0.92	4	7	1.7
elder	70	66	3.8 ⁽³⁾	4.8	420	0.92	22	38	1.8
obese	140		6.7	5.0 ⁽⁴⁾	610 ⁽⁵⁾	0.92	40	56	1.4

Gut microbiome can metabolize drugs differently



The *Tabula Sapiens* and other community projects offer reference expression data in healthy donors

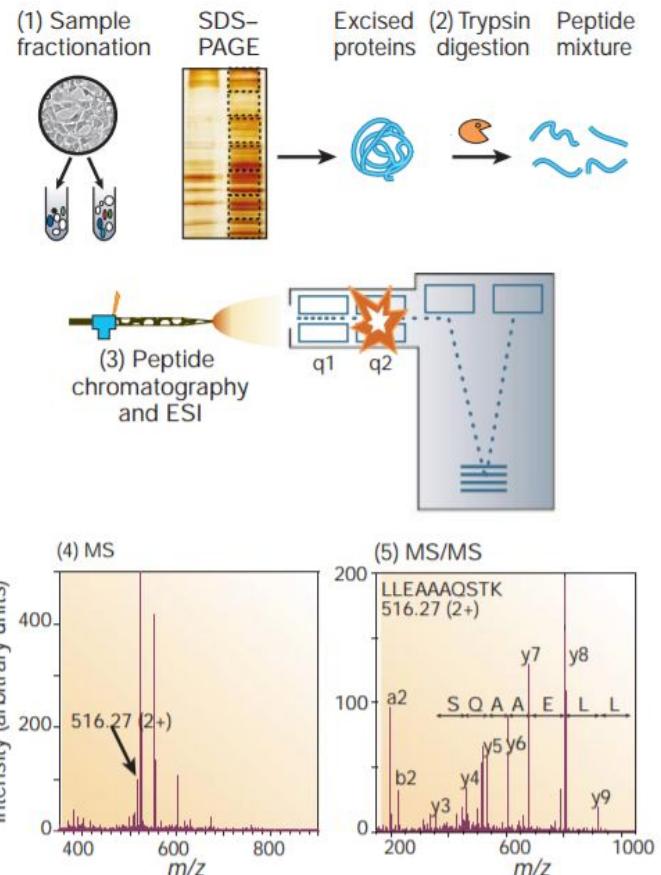


Left: the *Tabula Sapiens*. Right: Myeloid (MΦ=macrophages, Mo/monocytes, LAM=lipid-associated macrophages, DC=dendritic cells) gene expression

Understanding MoA of small molecules and antibodies with proteomics

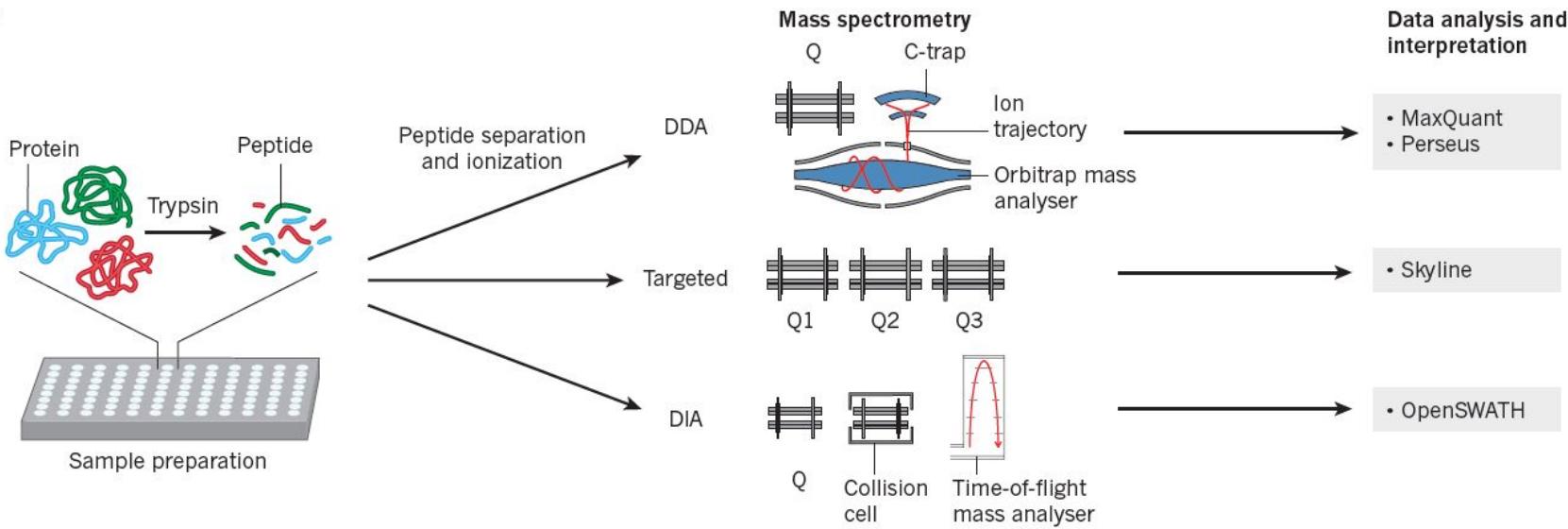
Mass-spectrometry based Proteomics

- **SDS-PAGE:** Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
- **ESI:** Electrospray ionization
- **q1/q2:** selection/collision/separation cells
- **MS:** Mass spectrometry
- **MS/MS:** tandem mass spectrometry

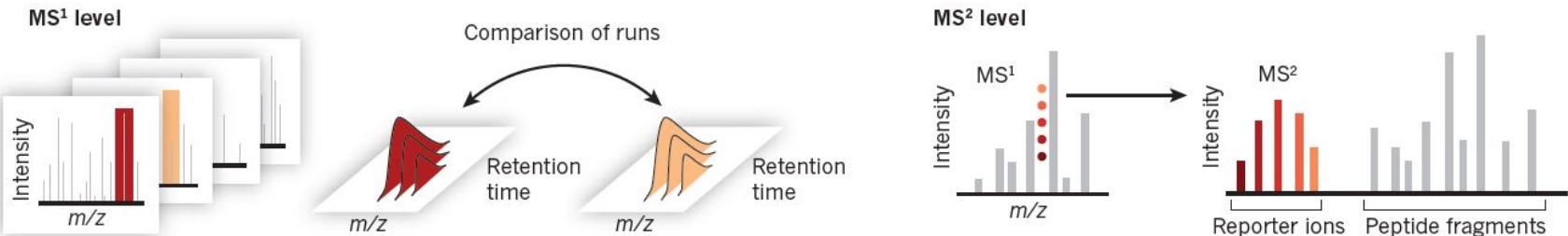


Mass-spectrometry based proteomics

a



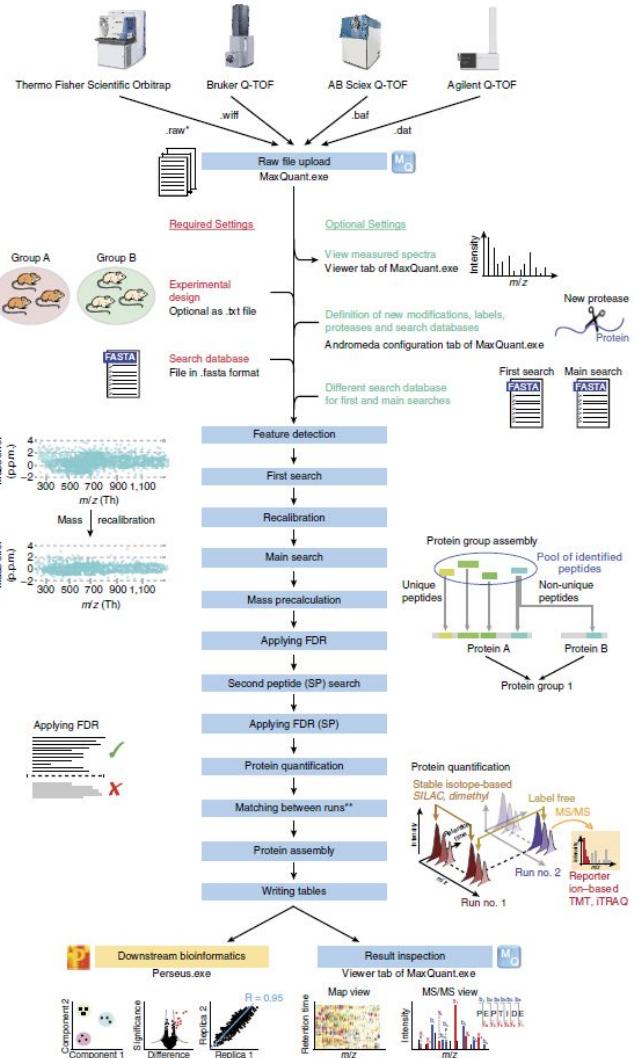
b Peptide quantification



We use mature software to handle MS data

Here is an example of *MaxQuant*.
Additional work needs to be done:

- Experiment design
- Statistical modelling
- Pathway and network analysis
- Integration with other data



Proteomics approaches for drug discovery

Affinity purification

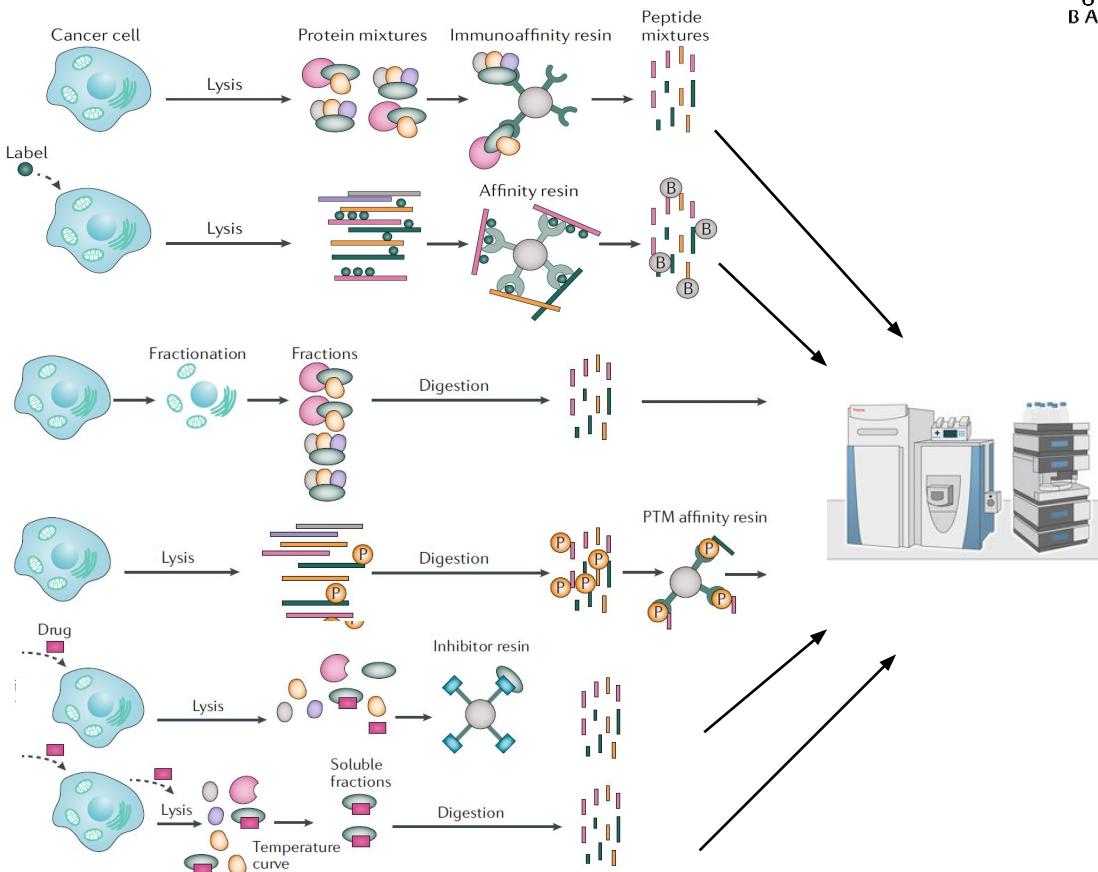
Proximity labelling

Organelle proteome profiling

Post-translational modification (PTM) profiling

Chemoaffinity enrichment

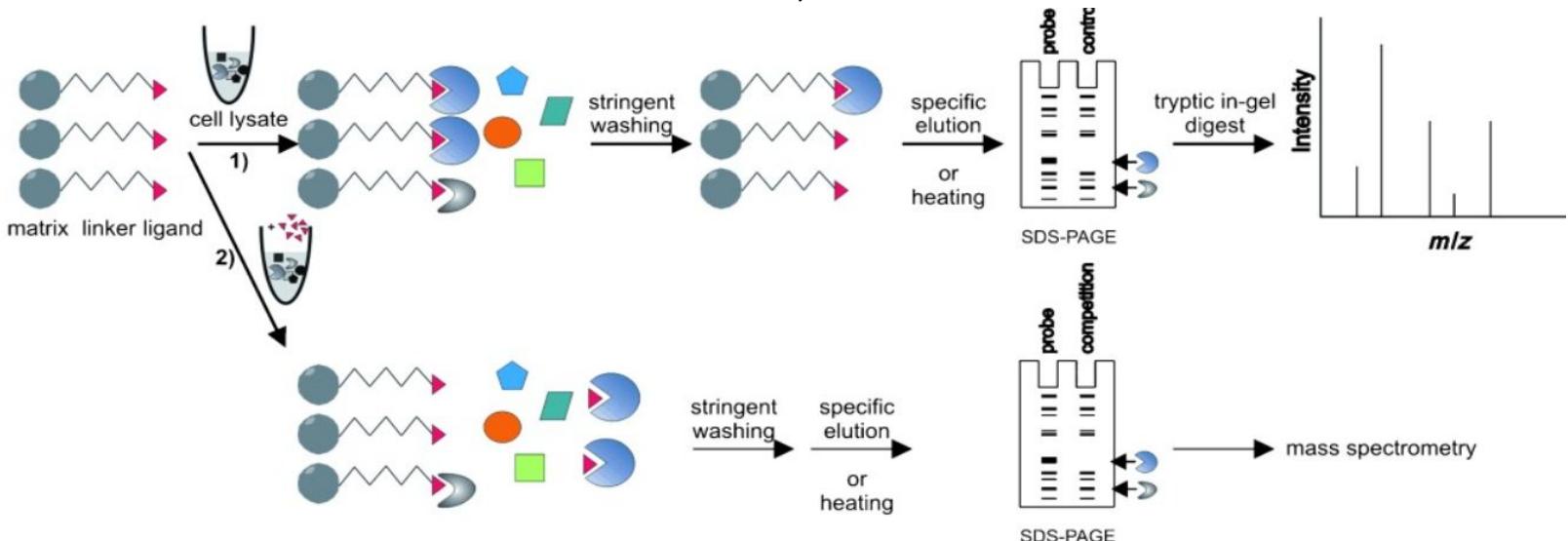
Thermal proteome profiling



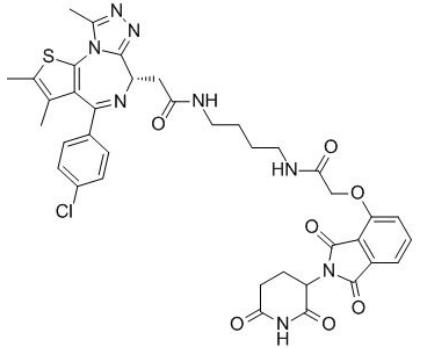
Example 1: Chemoproteomics for target ID



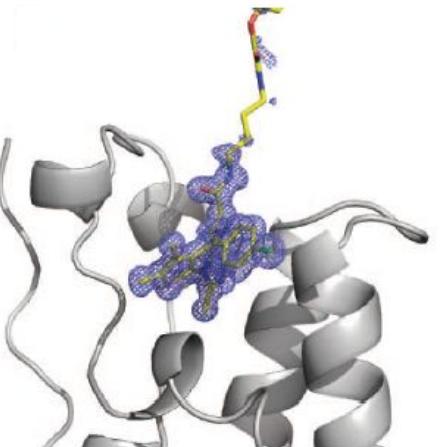
- Chemoproteomics methods are based on two principles: (1) **bait/prey** and (2) **competition**.
- Commonly used methods include affinity-based profiling (shown below), activity-based profiling, SILAC, etc.



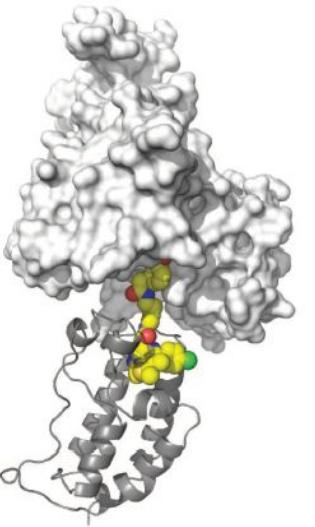
Example 2: Confirmation of selective degradation of protein target *in vivo*



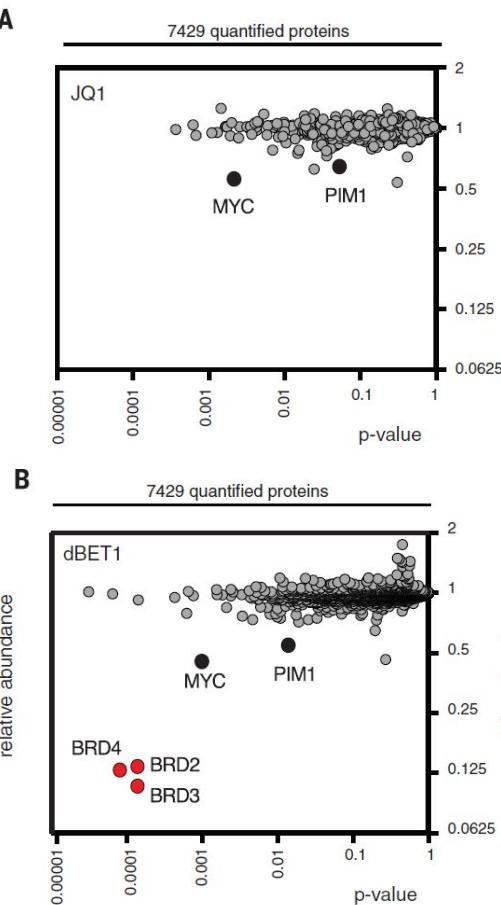
dBET1



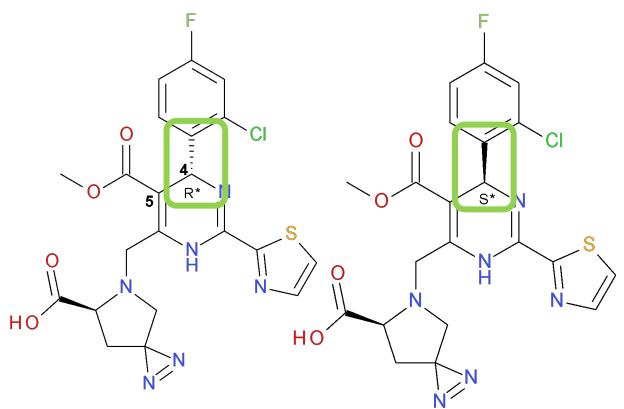
Crystal
structure of
dBET1 binding
to its target
BRD4



Docking of
dBET1-BRD4 to
DDB1-CRBN
structure



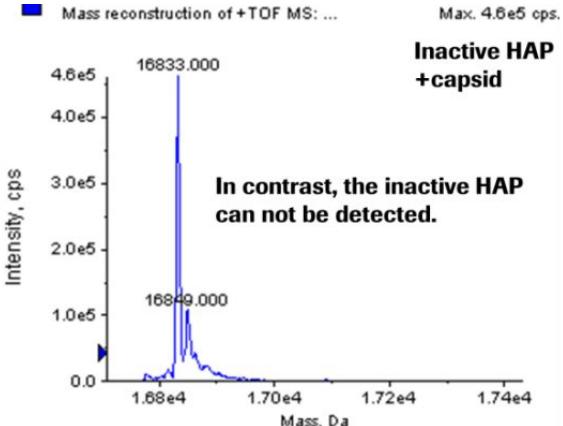
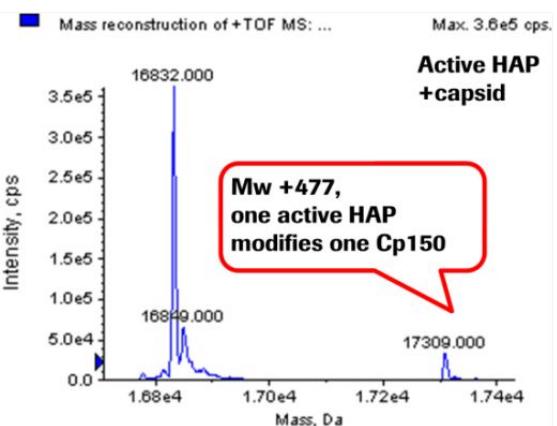
Example 3: photoaffinity labelling confirmed HBV capsid binding and mapped the small molecule binding pocket



RO-A
 EC_{50} : 0.040 μM
 IC_{50} : 0.47 μM

RO-B
 EC_{50} : >1 μM
 IC_{50} : >100 μM

+Cp150, UV, MS



Proteolytic digestion/LC-MS/MS identified labelling site **Y118 (Y=Tyrosine)** of HBV capsid protein. More photoaffinity probes identified labelling sites at **R127 (R=Arginine)** and **Y38**.

Conclusions

- We predict efficacy and safety profiles of drugs by studying the mechanism and mode of action (MoA).
- Molecular modelling, (single-cell) RNA sequencing, and proteomics based on mass spectrometry (MS) are essential tools for understanding MoA of small molecules and antibodies.

Offline activities of Module IV (optional)

Perform your own single-cell data analysis to get first-hand experience working with high-dimensional biological data.

- If you are new to the topic, please use [the PBMC tutorial of Scanpy \(python\)](#) or [the PBMC tutorial of Seurat \(R\)](#).
- If you have experience with such data already, checkout [the NBIS workshop on single-cell sequencing data analysis](#) to cover advanced topics such as spatial transcriptomics and trajectory inference.

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1. Figures: [Lumen Learning](#), [Exploring Nature](#), [National Geographic](#), [Platelet cells](#) (Graham Beards, CC-BY-SA 4.0), [Lymphocytes](#) (Nicolas Grandjean, CC-BY-SA 3.0), [Adipocytes](#) (Public Domain), [Hepatocytes](#) (CC-BY-NC 2.0), [Neurons and Glia](#) (Public Domain), [Blood](#) (CC 3.0), [Blood Cells](#) (By A. Rad and M. Häggström. CC-BY-SA 3.0 license), [A selective JAK3 inhibitor](#) (London Lab/Weizmann institute)
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Supplementary Information

Embryonic origins of tissues

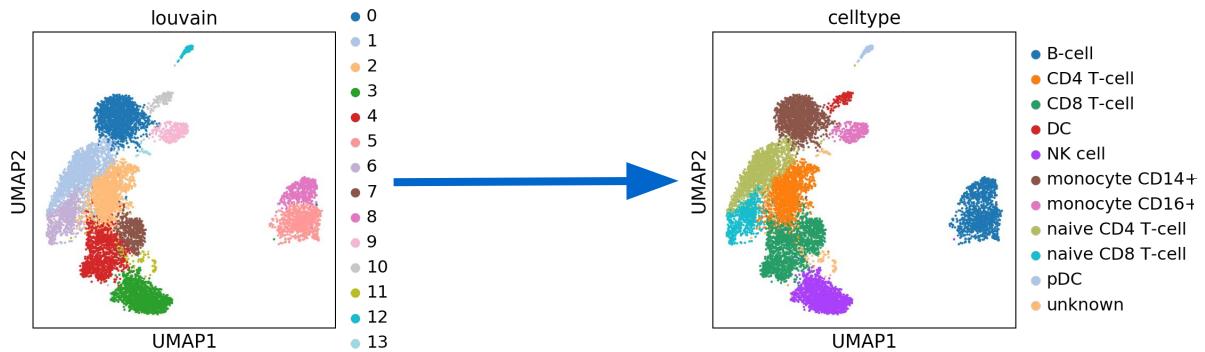
Germ Layer	Gives rise to:		
Ectoderm	Epidermis, glands on skin, some cranial bones, pituitary and adrenal medulla, the nervous system, the mouth between cheek and gums, the anus		
Mesoderm	Connective tissues proper, bone, cartilage, blood, endothelium of blood vessels, muscle, synovial membranes, serous membranes lining body cavities, kidneys, lining of gonads		
Endoderm	Lining of airways and digestive system except the mouth and distal part of digestive system (rectum and anal canal); glands (digestive glands, endocrine glands, adrenal cortex)		

An intern project: Cell type annotation

From unsupervised clustering and cluster based annotation



Luis Wyss
RAAN intern 2019



	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Label
Training Cell 1	10	50	0	12	4	Celltype A
Training Cell 2	8	45	78	3	23	Celltype B
Training Cell 3	14	55	78	65	55	Celltype B
Training Cell 4	78	12	13	9	58	Celltype A
Training Cell 5	45	23	65	98	11	Celltype C

To supervised annotation at single-cell level:

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
Cell 1	45	45	8	56	3
Cell 2	65	120	78	45	12
Cell 3	79	12	34	65	88
Cell 4	7	59	32	47	62

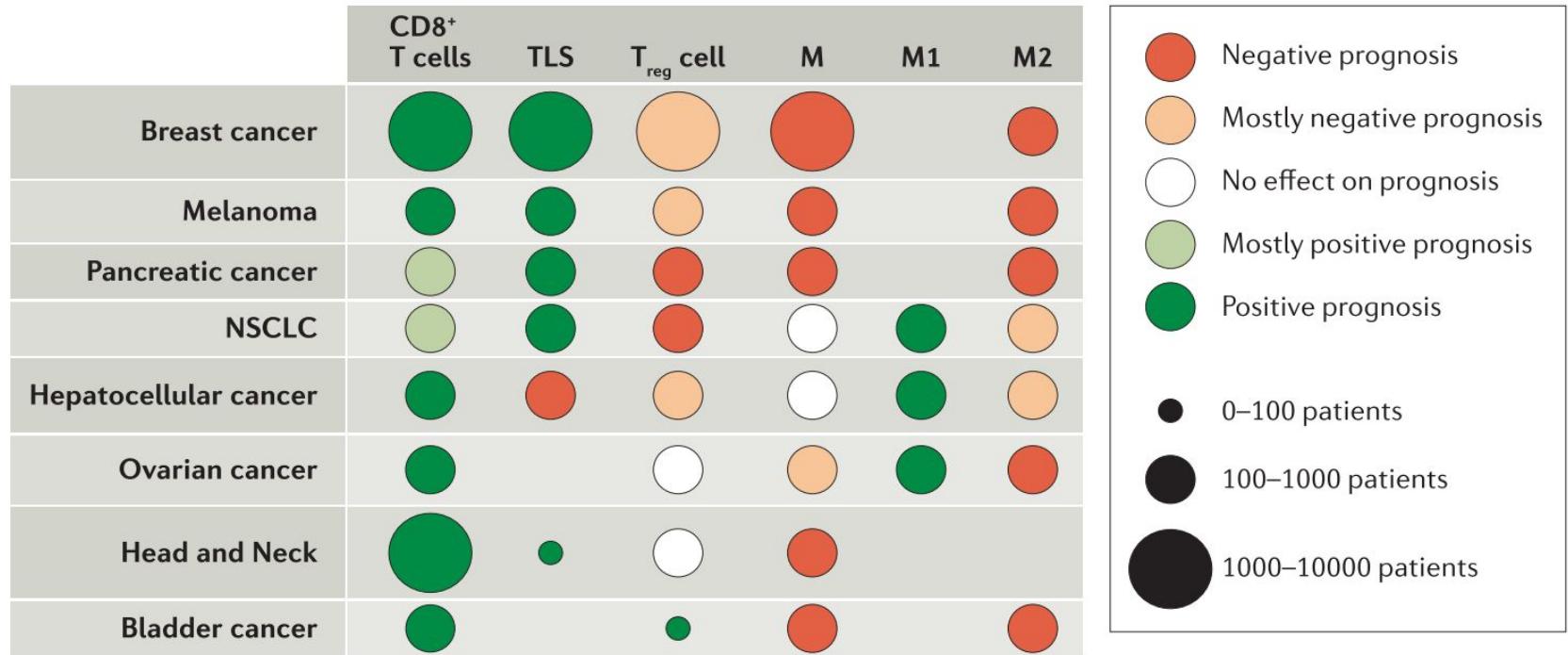


	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Prediction
Cell 1	45	45	8	56	3	Celltype A
Cell 2	65	120	78	45	12	Celltype B
Cell 3	79	12	34	65	88	Celltype C
Cell 4	7	59	32	47	62	Celltype B



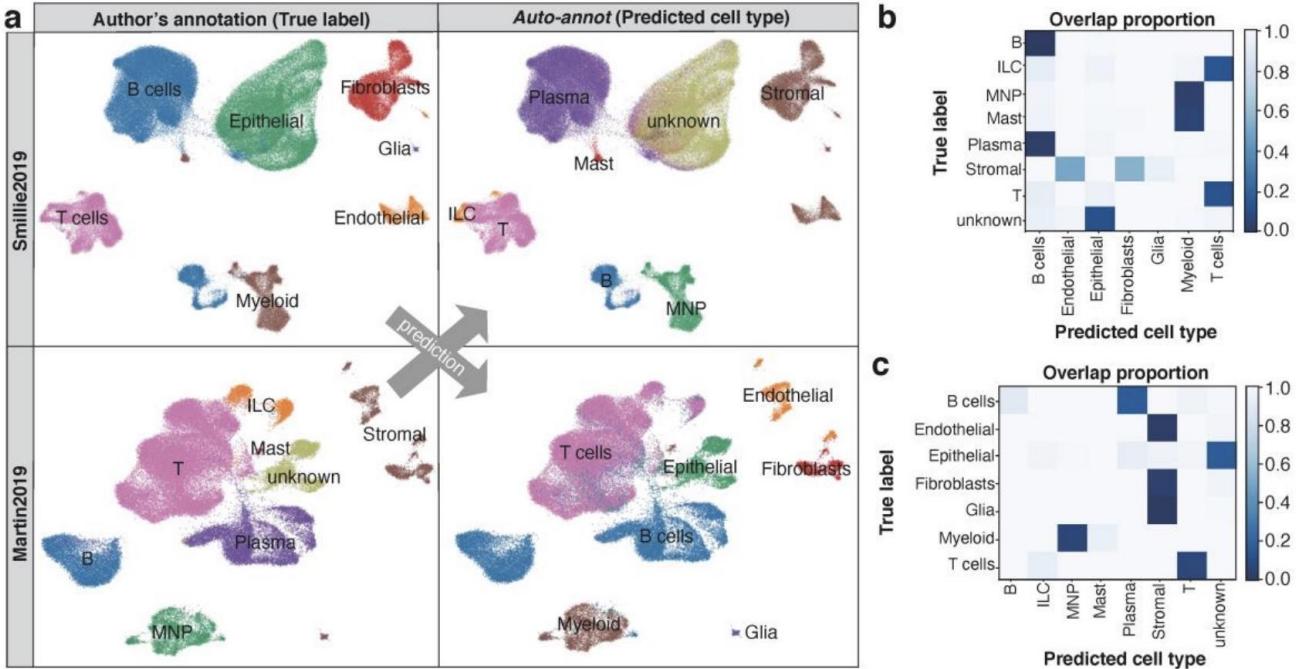
Advantages: (1) automation, (2) annotation independent from clustering, and (3) we can estimate the confidence of prediction

Abundance of immune cells in tumor microenvironments affect outcome



TLS: tertiary lymphoid structures; T_{reg}: regulatory T cells; M: macrophages; M1/M2: subtypes of macrophages

An example of Inflammatory Bowel Disease (IBD)



We observed Inconsistent cell type nomenclature across studies.
 Machine learning allows us compare and integrate multiple studies.

Proteomics enables the elucidation of protein relations in the protein communities

